

02-09-00

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02/08/00
490 U.S. PTO

Practitioner's Docket No. 46910-DIV-2

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Box Patent Application
Assistant Commissioner for Patents
Washington, D.C. 20231

551 U.S. PTO
09/499765
02/08/00

NEW APPLICATION TRANSMITTAL

Transmitted herewith for filing is the patent application of
Inventor(s): Y. Hayashi, et al.

WARNING: 37 C.F.R. § 1.41(a)(1) points out:

"(a) A patent is applied for in the name or names of the actual inventor or inventors.

(1) The inventorship of a nonprovisional application is that inventorship set forth in the oath or declaration as prescribed by § 1.63, except as provided for in § 1.53(d)(4) and § 1.63(d). If an oath or declaration as prescribed by § 1.63 is not filed during the pendency of a nonprovisional application, the inventorship is that inventorship set forth in the application papers filed pursuant to § 1.53(b), unless a petition under this paragraph accompanied by the fee set forth in § 1.17(i) is filed supplying or changing the name or names of the inventor or inventors."

For (title): COMPOSITION CONTAINING α -FODRIN OR α -FODRIN FRAGMENT PROTEIN

1. Type of Application

CERTIFICATION UNDER 37 C.F.R. 1.10*

(Express Mail label number is **mandatory**.)

(Express Mail certification is optional.)

I hereby certify that this correspondence and the documents referred to as attached therein are being deposited with the United States Postal Service on this date 2/8/00, in an envelope as "Express Mail Post Office to Addressee," mailing Label Number EL054597975US, addressed to the: Assistant Commissioner for Patents, Washington, D.C. 20231.

Annemarie Serrecchia
(type or print name of person mailing paper)

Annemarie Serrecchia
Signature of person mailing paper

WARNING: Certificate of mailing (first class) or facsimile transmission procedures of 37 C.F.R. 1.8 cannot be used to obtain a date of mailing or transmission for this correspondence.

***WARNING:** Each paper or fee filed by "Express Mail" **must** have the number of the "Express Mail" mailing label placed thereon prior to mailing. 37 C.F.R. 1.10(b).
"Since the filing of correspondence under § 1.10 without the Express Mail mailing label thereon is an oversight that can be avoided by the exercise of reasonable care, requests for waiver of this requirement will **not** be granted on petition." Notice of Oct. 24, 1996, 60 Fed. Reg. 56,439, at 56,442.

This new application is for a(n)

(check one applicable item below)

- ☒ Original (nonprovisional)
☐ Design
☐ Plant

WARNING: Do not use this transmittal for a completion in the U.S. of an International Application under 35 U.S.C. 371(c)(4), unless the International Application is being filed as a divisional, continuation or continuation-in-part application.

WARNING: Do not use this transmittal for the filing of a provisional application.

NOTE: If one of the following 3 items apply, then complete and attach **ADDED PAGES FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF A PRIOR U.S. APPLICATION CLAIMED** and a **NOTIFICATION IN PARENT APPLICATION OF THE FILING OF THIS CONTINUATION APPLICATION**.

- ☒ Divisional.
☐ Continuation.
☐ Continuation-in-part (C-I-P).

2. Benefit of Prior U.S. Application(s) (35 U.S.C. 119(e), 120, or 121)

NOTE: A nonprovisional application may claim an invention disclosed in one or more prior filed copending nonprovisional applications or copending international applications designating the United States of America. In order for a nonprovisional application to claim the benefit of a prior filed copending nonprovisional application or copending international application designating the United States of America, each prior application must name as an inventor at least one inventor named in the later filed nonprovisional application and disclose the named inventor's invention claimed in at least one claim of the later filed nonprovisional application in the manner provided by the first paragraph of 35 U.S.C. 112. Each prior application must also be:

- (i) An international application entitled to a filing date in accordance with PCT Article 11 and designating the United States of America; or
- (ii) Complete as set forth in § 1.51(b); or
- (iii) Entitled to a filing date as set forth in § 1.53(b) or § 1.53(d) and include the basic filing fee set forth in § 1.16; or
- (iv) Entitled to a filing date as set forth in § 1.53(b) and have paid therein the processing and retention fee set forth in § 1.21(l) within the time period set forth in § 1.53(f).

37 C.F.R. § 1.78(a)(1).

NOTE If the new application being transmitted is a divisional, continuation or a continuation-in-part of a parent case, or where the parent case is an International Application which designated the U.S., or benefit of a prior provisional application is claimed, then check the following item and complete and attach **ADDED PAGES FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF PRIOR U.S. APPLICATION(S) CLAIMED**.

WARNING: If an application claims the benefit of the filing date of an earlier filed application under 35 U.S.C. 120, 121 or 365(c), the 20-year term of that application will be based upon the filing date of the earliest U.S. application that

the application makes reference to under 35 U.S.C. 120, 121 or 365(c). (35 U.S.C. 154(a)(2) does not take into account, for the determination of the patent term, any application on which priority is claimed under 35 U.S.C. 119, 365(a) or 365(b).) For a c-i-p application, applicant should review whether any claim in the patent that will issue is supported by an earlier application and, if not, the applicant should consider canceling the reference to the earlier filed application. The term of a patent is not based on a claim-by-claim approach. See Notice of April 14, 1995, 60 Fed. Reg. 20,195, at 20,205.

WARNING: When the last day of pendency of a provisional application falls on a Saturday, Sunday, or Federal holiday within the District of Columbia, any nonprovisional application claiming benefit of the provisional application **must** be filed prior to the Saturday, Sunday, or Federal holiday within the District of Columbia. See 37 C.F.R. § 1.78(a)(3).

☒ The new application being transmitted claims the benefit of prior U.S. application(s).
Enclosed are ADDED PAGES FOR NEW APPLICATION TRANSMITTAL WHERE
BENEFIT OF PRIOR U.S. APPLICATION(S) CLAIMED.

3. Papers Enclosed

A. Required for Filing Date under 37 C.F.R. § 1.53(b) (Regular) or 37 C.F.R. § 1.153 (Design) Application

34 Pages of Specification plus cover sheet
3 Pages of Claims
4 Sheets of Drawing

WARNING: **DO NOT** submit original drawings. A high quality copy of the drawings should be supplied when filing a patent application. The drawings that are submitted to the Office must be on strong, white, smooth, and non-shiny paper and meet the standards according to § 1.84. If corrections to the drawings are necessary, they should be made to the original drawing and a high-quality copy of the corrected original drawing then submitted to the Office. Only one copy is required or desired. For comments on proposed then-new 37 C.F.R. 1.84, see Notice of March 9, 1988 (1990 O.G. 57-62).

NOTE: "Identifying indicia, if provided, should include the application number or the title of the invention, inventor's name, docket number (if any), and the name and telephone number of a person to call if the Office is unable to match the drawings to the proper application. This information should be placed on the back of each sheet of drawing a minimum distance of 1.5 cm. (5/8 inch) down from the top of the page. . ." 37 C.F.R. § 1.84(c)).

(complete the following, if applicable)

☐ The enclosed drawing(s) are photograph(s), and there is also attached a "PETITION TO ACCEPT PHOTOGRAPH(S) AS DRAWING(S)." 37 C.F.R. § 1.84(b).

☒ Formal
☐ Informal

B. Other Papers Enclosed

3 Pages of declaration and power of attorney
1 Pages of Abstract
Other

4. Additional Papers Enclosed

- ☐ Amendment to claims
- ☐ Cancel in this applications claims _____ before calculating the filing fee. (At least one original independent claim must be retained for filing purposes.)
- ☐ Add the claims shown on the attached amendment. (Claims added have been numbered consecutively following the highest numbered original claims.)
- ☒ Preliminary Amendment
- ☒ Information Disclosure Statement (37 C.F.R. § 1.98)
- ☒ Form PTO-1449 (PTO/SB/08A and 08B)
- ☐ Citations
- ☐ Declaration of Biological Deposit
- ☐ Submission of "Sequence Listing," computer readable copy and/or amendment pertaining thereto for biotechnology invention containing nucleotide and/or amino acid sequence.
- ☐ Authorization of Attorney(s) to Accept and Follow Instructions from Representative
- ☐ Special Comments
- ☒ Other - Transfer of Sequence Listing and Sequence Listing (page 33 of application)

5. Declaration or Oath (including power of attorney)

NOTE: *A newly executed declaration is not required in a continuation or divisional application provided the prior nonprovisional application contained a declaration as required, the application being filed is by all or fewer than all the inventors named in the prior application, there is no new matter in the application being filed, and a copy of the executed declaration filed in the prior application (showing the signature or an indication thereon that it was signed) is submitted. The copy must be accompanied by a statement requesting deletion of the names of person(s) who are not inventors of the application being filed. If the declaration in the prior application was filed under § 1.47 then a copy of that declaration must be filed accompanied by a copy of the decision granting § 1.47 status or, if a nonsigning person under § 1.47 has subsequently joined in a prior application, then a copy of the subsequently executed declaration must be filed See 37 C.F.R. § 1.63(d)(1)-(3).*

NOTE: *A declaration filed to complete an application must be executed, identify the specification to which it is directed, identify each inventor by full name, including the family name, and at least one given name without abbreviation together with any other given name or initial, and the residence, post office address and country of citizenship of each inventor, and state whether the inventor is a sole or joint inventor. 37 C.F.R. § 1.63(a)(1)-(4).*

☒ Enclosed

Executed by

(check all applicable boxes)

- ☒ inventor(s).
- ☐ legal representative of inventor(s). 37 C.F.R. § 1.42 or 1.43.
- ☐ joint inventor or person showing a proprietary interest on behalf of inventor who refused to sign or cannot be reached.

☐ This is the petition required by 37 C.F.R. § 1.47 and the statement required by 37 C.F.R. § 1.47 is also attached. See item 13 below for fee.

☐ Not Enclosed.

NOTE: *Where the filing is a completion in the U.S. of an International Application, or where the completion of the U.S. application contains subject matter in addition to the International Application, the application may be treated as a continuation or continuation-in-part, as the case may be, utilizing ADDED PAGE FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF PRIOR U.S. APPLICATION CLAIMED.*

☐ Application is made by a person authorized under 37 C.F.R. 1.41(c) on behalf of all the above named inventor(s).

(The declaration or oath, along with the surcharge required by 37 C.F.R. § 1.16(e), can be filed subsequently).

☐ Showing that the filing is authorized.
(not required unless called into question. 37 C.F.R. § 1.41(d))

6. Inventorship Statement

WARNING: *If the named inventors are each not the inventors of all the claims an explanation, including the ownership of the various claims at the time the last claimed invention was made, should be submitted.*

The inventorship for all the claims in this application are:

☒ The same.

or

☐ Not the same. An explanation, including the ownership of the various claims at the time the last claimed invention was made,

☐ is submitted.

☐ will be submitted.

7. Language

NOTE: *An application including a signed oath or declaration may be filed in a language other than English. An English translation of the non-English language application and the processing fee of \$130.00 required by 37 C.F.R. § 1.17(k) is required to be filed with the application, or within such time as may be set by the Office. 37 C.F.R. § 1.52(d).*

☒ English

☐ Non-English

☐ The attached translation includes a statement that the translation is accurate. 37 C.F.R. § 1.52(d).

8. Assignment

☐ An assignment of the invention to _____

☐ is attached. A separate ☐ "COVER SHEET FOR ASSIGNMENT (DOCUMENT) ACCOMPANYING NEW PATENT APPLICATION" or ☐ FORM PTO 1595 is also attached.

☐ will follow.

NOTE: "If an assignment is submitted with a new application, send two separate letters—one for the application and one for the assignment" Notice of May 4, 1990 (1114 O.G. 77-78).

WARNING: A newly executed "STATEMENT UNDER 37 C.F.R. § 3.73(b)" must be filed when a continuation-in-part application is filed by an assignee. Notice of April 30, 1993, 1150 O.G. 62-64.

9. Certified Copy

Certified copy(ies) of application(s)

Country	Appln. no.	Filed
Country	Appln. no.	Filed
Country	Appln. no.	Filed

from which priority is claimed

☐ is (are) attached.

☐ will follow.

NOTE: The foreign application forming the basis for the claim for priority must be referred to in the oath or declaration. 37 C.F.R. § 1.55(a) and 1.63.

NOTE: This item is for any foreign priority for which the application being filed directly relates. If any parent U.S. application or International Application from which this application claims benefit under 35 U.S.C. 120 is itself entitled to priority from a prior foreign application, then complete item 18 on the ADDED PAGES FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF PRIOR U.S. APPLICATION(S) CLAIMED.

10. Fee Calculation (37 C.F.R. § 1.16)

A. ☒ Regular application

CLAIMS AS FILED

Claims	Number Filed	Basic Fee Allowance	Number Extra	Rate	Basic Fee 37 C.F.R. §1.16(a) \$690.00
Total Claims (37 C.F.R. §1.16(c))	6	- 20 =	x	\$ 18.00	
Independent Claims (37 C.F.R. § 1.16(b))	1	- 3 =	x	\$ 78.00	
Multiple Dependent Claim(s), if any (37 C.F.R. § 1.16(d))			+	\$260.00	

- ☒ [X] Amendment cancelling extra claims is enclosed.
☐ [] Amendment deleting multiple-dependencies is enclosed.
☐ [] Fee for extra claims is not being paid at this time.

NOTE. *If the fees for extra claims are not paid on filing they must be paid or the claims cancelled by amendment, prior to the expiration of the time period set for response by the Patent and Trademark Office in any notice of fee deficiency. 37 C.F.R. § 1.16(d).*

Filing Fee Calculation \$ 690.00

- B.** ☐ [] Design application
(\$310.00—37 C.F.R. § 1.16(f))

Filing Fee Calculation \$ _____

- C.** ☐ [] Plant application
(\$480.00—37 C.F.R. § 1.16(g))

Filing Fee Calculation \$ _____

11. Small Entity Statement(s)

- ☐ [] Statement(s) that this is a filing by a small entity under 37 C.F.R. §§ 1.9 and 1.27 is (are) attached.

WARNING: *"Status as a small entity must be specifically established in each application or patent in which the status is available and desired. Status as a small entity in one application or patent does not affect any other application or patent,*

including applications or patents which are directly or indirectly dependent upon the application or patent in which the status has been established. The refiling of an application under § 1.53 as a continuation, division, or continuation-in-part (including a continued prosecution application under § 1.53(d)), or the filing of a reissue application requires a new determination as to continued entitlement to small entity status for the continuing or reissue application. A nonprovisional application claiming benefit under 35 U.S.C. 119(e), 120, 121, or 365(c) of a prior application, or a reissue application may rely on a statement filed in the prior application or in the patent if the nonprovisional application or the reissue application includes a reference to the statement in the prior application or in the patent or includes a copy of the statement in the prior application or in the patent and status as a small entity is still proper and desired. The payment of the small entity basic statutory filing fee will be treated as such a reference for purposes of this section." 37 C.F.R. § 1.28(a)(2).

(complete the following, if applicable)

☐ Status as a small entity was claimed in prior application
_____/_____, filed on _____ from which benefit is being
claimed for this application under:

35 U.S.C. § ☐ 119(e),
☐ 120,
☐ 121,
☐ 365(c),

and which status as a small entity is still proper and desired.

☐ A copy of the statement in the prior application is included.

Filing Fee Calculation (50% of A, B or C above) \$ _____

NOTE: Any excess of the full fee paid will be refunded if a small entity status is established refund request are filed within 2 months of the date of timely payment of a full fee. The two-month period is not extendable under § 1.136. 37 C.F.R. § 1.28(a).

12. Request for International-Type Search (37 C.F.R. § 1.104(d))

(complete, if applicable)

☐ Please prepare an international-type search report for this application at the time when national examination on the merits takes place.

13. Fee Payment Being Made at This Time

☐ Not Enclosed

☐ No filing fee is to be paid at this time.
(This and the surcharge required by 37 C.F.R. § 1.16(e) can be paid subsequently.)

☒ Enclosed

☒ Filing fee \$ _____ 690.00

- ☐ Recording assignment
(\$40.00; 37 C.F.R. § 1.21(h))
(See attached "COVER SHEET FOR
ASSIGNMENT ACCOMPANYING NEW
APPLICATION.") \$ _____
- ☐ Petition fee for filing by other
than all the inventors or person
on behalf of the inventor where
inventor refused to sign or cannot
be reached
(\$130.00; 37 C.F.R. §§ 1.47 and 1.17(i)) \$ _____
- ☐ For processing an application with a
specification in a non-English language
(\$130.00; 37 C.F.R. §§ 1.52(d) and 1.17(k)) \$ _____
- ☐ Processing and retention fee
(\$130.00; 37 C.F.R. §§ 1.53(d) and 1.21(l)) \$ _____
- ☐ Fee for international-type search report
(\$40.00; 37 C.F.R. § 1.21(e)) \$ _____

NOTE: 37 C.F.R. § 1.21(l) establishes a fee for processing and retaining any application that is abandoned for failing to complete the application pursuant to 37 C.F.R. § 1.53(f) and this, as well as the changes to 37 C.F.R. § 1.53 and 1.78(a)(1), indicate that in order to obtain the benefit of a prior U.S. application, either the basic filing fee must be paid, or the processing and retention fee of § 1.21(l) must be paid, within 1 year from notification under § 53(f).

Total Fees Enclosed \$ 690.00

14. Method of Payment of Fees

- ☒ Check in the amount of \$ 690.00 .
- ☐ Charge Account No. _____ in the amount of \$ _____.
A duplicate of this transmittal is attached.

NOTE: Fees should be itemized in such a manner that it is clear for which purpose the fees are paid. 37 C.F.R. § 1.22(b).

15. Authorization to Charge Additional Fees

WARNING: If no fees are to be paid on filing, the following items should not be completed.

WARNING: Accurately count claims, especially multiple dependent claims, to avoid unexpected high charges, if extra claim charges are authorized.

☐ The Commissioner is hereby authorized to charge the following additional fees by this paper and during the entire pendency of this application to Account No. 04-1105.

☒ 37 C.F.R. § 1.16(a), (f) or (g) (filing fees)

☐ 37 C.F.R. § 1.16(b), (c) and (d) (presentation of extra claims)

NOTE: Because additional fees for excess or multiple dependent claims not paid on filing or on later presentation must only be paid or these claims cancelled by amendment prior to the expiration of the time period set for response by the PTO in any notice of fee deficiency (37 C.F.R. § 1.16(d)), it might be best not to authorize the PTO to charge additional claim fees, except possibly when dealing with amendments after final action.

☒ 37 C.F.R. § 1.16(e) (surcharge for filing the basic filing fee and/or declaration on a date later than the filing date of the application)

☒ 37 C.F.R. § 1.17(a)(1)-(5) (extension fees pursuant to § 1.136(a).

☒ 37 C.F.R. § 1.17 (application processing fees)

NOTE: "A written request may be submitted in an application that is an authorization to treat any concurrent or future reply, requiring a petition for an extension of time under this paragraph for its timely submission, as incorporating a petition for extension of time for the appropriate length of time. An authorization to charge all required fees, fees under § 1.17, or all required extension of time fees will be treated as a constructive petition for an extension of time in any concurrent or future reply requiring a petition for an extension of time under this paragraph for its timely submission. Submission of the fee set forth in § 1.17(a) will also be treated as a constructive petition for an extension of time in any concurrent reply requiring a petition for an extension of time under this paragraph for its timely submission." 37 C.F.R. § 1.136(a)(3).

☐ 37 C.F.R. § 1.18 (issue fee at or before mailing of Notice of Allowance, pursuant to 37 C.F.R. § 1.311(b))

NOTE: Where an authorization to charge the issue fee to a deposit account has been filed before the mailing of a Notice of Allowance, the issue fee will be automatically charged to the deposit account at the time of mailing the notice of allowance. 37 C.F.R. § 1.311(b)).

NOTE: 37 C.F.R. § 1.28(b) requires "Notification of any change in status resulting in loss of entitlement to small entity status must be filed in the application . . . prior to paying, or at the time of paying, . . . issue fee." From the wording of 37 C.F.R. § 1.28(b), (a) notification of change of status must be made even if the fee is paid as "other than a small entity" and (b) no notification is required if the change is to another small entity.

16. Instructions as to Overpayment

NOTE: "... Amounts of twenty-five dollars or less will not be returned unless specifically requested within a reasonable time, nor will the payer be notified of such amounts; amounts over twenty-five dollars may be returned by check or, if requested, by credit to a deposit account." 37 C.F.R. § 1.26(a).

☒ Credit Account No. - 04-1105.

☐ Refund

Reg. No. 38,227

Tel. No.: (617)

Customer No.:

Cara Z. Lowen Feb 8, 2000
SIGNATURE OF PRACTITIONER

Cara Z. Lowen

(type or print name of practitioner)

Dike, Bronstein, Roberts & Cushman, LLP

130 Water Street

P.O. Address

Boston, MA 02109

☒ **Incorporation by reference of added pages**

(check the following item if the application in this transmittal claims the benefit of prior U.S. application(s) (including an international application entering the U.S. stage as a continuation, divisional or C-I-P application) and complete and attach the ADDED PAGES FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF PRIOR U.S. APPLICATION(S) CLAIMED)

☒ Plus Added Pages for New Application Transmittal Where Benefit of Prior U.S. Application(s) Claimed

Number of pages added 5

☐ Plus Added Pages for Papers Referred to in Item 4 Above

Number of pages added _____

☐ Plus added pages deleting names of inventor(s) named on prior application(s) who is/are no longer inventor(s) of the subject matter claimed in this application.

Number of pages added _____

☐ Plus "Assignment Cover Letter Accompanying New Application"

Number of pages added _____

☐ **Statement Where No Further Pages Added**

(if no further pages form a part of this Transmittal, then end this Transmittal with this page and check the following item)

☐ This transmittal ends with this page.

ADDED PAGES FOR APPLICATION TRANSMITTAL WHERE BENEFIT OF PRIOR U.S. APPLICATION(S) CLAIMED

NOTE See 37 C.F.R. § 1.78

17. Relate Back

WARNING: *If an application claims the benefit of the filing date of an earlier filed application under 35 U.S.C. 120, 121 or 365(c), the 20-year term of that application will be based upon the filing date of the earliest U.S. application that the application makes reference to under 35 U.S.C. 120, 121 or 365(c). (35 U.S.C. 154(a)(2) does not take into account, for the determination of the patent term, any application on which priority is claimed under 35 U.S.C. 119, 365(a) or 365(b).) For a c-i-p application, applicant should review whether any claim in the patent that will issue is supported by an earlier application and, if not, the applicant should consider canceling the reference to the earlier filed application. The term of a patent is not based on a claim-by-claim approach. See Notice of April 14, 1995, 60 Fed. Reg. 20,195, at 20,205.*

(complete the following, if applicable)

☒ Amend the specification by inserting, before the first line, the following sentence:

A. 35 U.S.C. 119(e)

NOTE: *"Any nonprovisional application claiming the benefit of one or more prior filed copending provisional applications must contain or be amended to contain in the first sentence of the specification following the title a reference to each such prior provisional application, identifying it as a provisional application, and including the provisional application number (consisting of series code and serial number)." 37 C.F.R. § 1.78(a)(4).*

☐ "This application claims the benefit of U.S. Provisional Application(s) No(s):

APPLICATION NO(S):

FILING DATE

_____ / _____	_____ "
_____ / _____	_____ "
_____ / _____	_____ "

B. 35 U.S.C. 120, 121 and 365(c)

NOTE. *"Except for a continued prosecution application filed under § 1.53(d), any nonprovisional application claiming the benefit of one or more prior filed copending nonprovisional applications or international applications designating the United States of America must contain or be amended to contain in the first sentence of the specification following the title a reference to each such prior application, identifying it by application number (consisting of the series code and serial number) or international application number and international filing date and indicating the relationship of the applications. . . . Cross-references to other related applications may be made when appropriate." (See § 1.14(a)). 37 C.F.R. § 1.78(a)(2).*

☒ "This application is a

☐ continuation

☐ continuation-in-part

☒ divisional

of copending application(s)

☒ application number 09/076,938, filed on May 13, 1998, which is a divisional of application number 08/736,434, filed on October 24, 1996 (now abandoned).

☐ International Application _____ filed on _____ now which designated the U.S.”

NOTE: The proper reference to a prior filed PCT application that entered the U.S. national phase is the U.S. serial number and the filing date of the PCT application that designated the U.S.

NOTE: (1) Where the application being transmitted adds subject matter to the International Application, then the filing can be as a continuation-in-part or (2) if it is desired to do so for other reasons then the filing can be as a continuation.

NOTE: The deadline for entering the national phase in the U.S. for an international application was clarified in the Notice of April 28, 1987 (1079 O.G. 32 to 46) as follows.

“The Patent and Trademark Office considers the International application to be pending until the 22nd month from the priority date if the United States has been designated and no Demand for International Preliminary Examination has been filed prior to the expiration of the 19th month from the priority date and until the 32nd month from the priority date if a Demand for International Preliminary Examination which elected the United States of America has been filed prior to the expiration of the 19th month from the priority date, provided that a copy of the international application has been communicated to the Patent and Trademark Office within the 20 or 30 month period respectively. If a copy of the international application has not been communicated to the Patent and Trademark Office within the 20 or 30 month period respectively, the international application becomes abandoned as to the United States 20 or 30 months from the priority date respectively. These periods have been placed in the rules as paragraph (h) of § 1.494 and paragraph (i) of § 1.495. A continuing application under 35 U.S.C. 365(c) and 120 may be filed anytime during the pendency of the international application.”

☐ “The nonprovisional application designated above, namely application _____ / _____, filed _____, claims the benefit of U.S. Provisional Application(s) No(s).:

APPLICATION NO(S).:

FILING DATE

_____ / _____	_____ ”
_____ / _____	_____ ”
_____ / _____	_____ ”

☐ Where more than one reference is made above please combine all references into one sentence.

18. Relate Back—35 U.S.C. 119 Priority Claim for Prior Application

The prior U.S. application(s), including any prior International Application designating the U.S., identified above in item 17B, in turn itself claim(s) foreign priority(ies) as follows:

<u>Japan</u>	<u>100765/1996</u>	<u>April 23, 1996</u>
Country	Appln. no.	Filed

The certified copy(ies) has (have)

☒ [X] been filed on 9/11/97, in prior application 08/736,434,
which was filed on 10/24/96.

☐ [] is (are) attached.

WARNING: *The certified copy of the priority application that may have been communicated to the PTO by the International Bureau may not be relied on without any need to file a certified copy of the priority application in the continuing application. This is so because the certified copy of the priority application communicated by the International Bureau is placed in a folder and is not assigned a U.S. serial number unless the national stage is entered. Such folders are disposed of if the national stage is not entered. Therefore, such certified copies may not be available if needed later in the prosecution of a continuing application. An alternative would be to physically remove the priority documents from the folders and transfer them to the continuing application. The resources required to request transfer, retrieve the folders, make suitable record notations, transfer the certified copies, enter and make a record of such copies in the Continuing Application are substantial. Accordingly, the priority documents in folders of international applications that have not entered the national stage may not be relied on. Notice of April 28, 1987 (1079 O.G. 32 to 46).*

19. Maintenance of Copendency of Prior Application

NOTE: *The PTO finds it useful if a copy of the petition filed in the prior application extending the term for response is filed with the papers constituting the filing of the continuation application. Notice of November 5, 1985 (1060 O.G. 27).*

A. ☐ [] Extension of time in prior application

*(This item **must** be completed and the papers filed **in the prior application**, if the period set in the prior application has run.)*

☐ [] A petition, fee and response extends the term in the pending **prior** application until _____.

☐ [] A **copy** of the petition filed in prior application is attached.

B. ☐ [] Conditional Petition for Extension of Time in Prior Application

(complete this item, if previous item not applicable)

☐ [] A conditional petition for extension of time is being filed in the pending **prior** application.

☐ [] A **copy** of the conditional petition filed in the prior application is attached.

20. Further Inventorship Statement Where Benefit of Prior Application(s) Claimed

(complete applicable item (a), (b) and/or (c) below)

- (a) ☒ This application discloses and claims only subject matter disclosed in the prior application whose particulars are set out above and the inventor(s) in this application are

☒ the same.

☐ less than those named in the prior application. It is requested that the following inventor(s) identified for the prior application be deleted:

(type name(s) of inventor(s) to be deleted)

- (b) ☐ This application discloses and claims additional disclosure by amendment and a new declaration or oath is being filed. With respect to the prior application, the inventor(s) in this application are

☐ the same.

☐ the following additional inventor(s) have been added:

(type name(s) of inventor(s) to be deleted)

- (c) ☒ The inventorship for all the claims in this application are

☒ the same.

☐ not the same. An explanation, including the ownership of the various claims at the time the last claimed invention was made

☐ is submitted.

☐ will be submitted.

21. Abandonment of Prior Application *(if applicable)*

- ☐ Please abandon the prior application at a time while the prior application is pending, or when the petition for extension of time or to revive in that application is granted, and when this application is granted a filing date, so as to make this application copending with said prior application.

NOTE: According to the Notice of May 13, 1983 (103, TMOG 6-7), the filing of a continuation or continuation-in-part application is a proper response with respect to a petition for extension of time or a petition to revive and should include the express abandonment of the prior application conditioned upon the granting of the petition and the granting of a filing date to the continuing application.

22. Petition for Suspension of Prosecution for the Time Necessary to File an Amendment

WARNING: *"The claims of a new application may be finally rejected in the first Office action in those situations where (1) the new application is a continuing application of, or a substitute for, an earlier application, and (2) all the claims of the new application (a) are drawn to the same invention claimed in the earlier application, and (b) would have been properly finally rejected on the grounds of art of record in the next Office action if they had been entered in the earlier application." MPEP, § 706.07(b), 6th ed., rev.2*

NOTE: *Where it is possible that the claims on file will give rise to a first action final for this continuation application and for some reason an amendment cannot be filed promptly (e.g., experimental data is being gathered) it may be desirable to file a petition for suspension of prosecution for the time necessary.*

(check the next item, if applicable)

☐ There is provided herewith a Petition To Suspend Prosecution for the Time Necessary to File An Amendment (New Application Filed Concurrently)

23. Small Entity (37 CFR § 1.28(a))

☐ Applicant has established small entity status by the filing of a statement in parent application / _____ on _____.

☐ A copy of the statement previously filed is included.

WARNING: See 37 CFR § 1.28(a)

24. NOTIFICATION IN PARENT APPLICATION OF THIS FILING

☐ A notification of the filing of this
(check one of the following)

☐ continuation

☐ continuation-in-part

☐ divisional

is being filed in the parent application, from which this application claims priority under 35 U.S.C. § 120.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT: Hayashi, et al.

U.S.S.N.: Divisional of
09/076,938

Art Unit: Unassigned

FILED: Herewith

Examiner: Unassigned

FOR: COMPOSITION CONTAINING α -FODRIN OR α -FODRIN
FRAGMENT PROTEIN

ASSISTANT COMMISSIONER FOR PATENTS
WASHINGTON, DC 20231

.....
CERTIFICATE OF MAILING

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By: Annemarie Serrecchia
Annemarie Serrecchia

.....
PRELIMINARY AMENDMENT

In the Claims:

Please cancel claims 1-13 and 20-23 without prejudice.

REMARKS

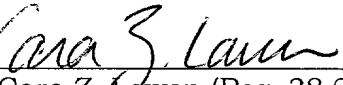
Applicants have preliminary cancelled claims 1-13 and 20-24 without prejudice.

Applicants respectfully submit that the present application is in condition for allowance. The Examiner is invited and encouraged to telephone the undersigned with any concerns in furtherance of the present application.

Respectfully submitted,

DIKE, BRONSTEIN, ROBERTS
& CUSHMAN, LLP

Date: Feb 8, 2000



Cara Z. Lowen (Reg. 38,227)
Attorney for Applicant
130 Water Street
Boston, MA 02109
(617) 523-3400

133972

PATENT
Case No.: 46,910

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
APPLICATION FOR UNITED STATES LETTERS PATENT

INVENTOR(S): YOSHIO HAYASHI and HIROMU SUGINO

TITLE: COMPOSITION CONTAINING α -fodrin or α -fodrin
FRAGMENT PROTEIN

ATTORNEY: David G. Conlin, Esq.
David S. Resnick, Esq.
Dike, Bronstein, Roberts & Cushman, LLP
130 Water Street
Boston, MA 02109
617-523-3400

DESCRIPTIONCOMPOSITION CONTAINING α -FODRIN OR α -FODRIN
FRAGMENT PROTEINTechnical Field

5 The present invention relates to new uses for α -fodrin and α -fodrin fragment proteins.

Background Art

10 Sjögren's syndrome is said to be an autoimmune disease whose cardinal manifestation is a sicca syndrome associated with keratoconjunctivitis sicca and chronic sialadenitis but the mechanisms of its onset remain to be elucidated.

15 Diagnosed as Sjögren's syndrome is either a glandular (primary) syndrome which is confined to the lacrimal and salivary glands and clinically characterized by the so-called dry eye/dry mouth symptom or an extraglandular (secondary) syndrome characterized by a broad spectrum of generalized symptoms involving the liver, lung, thyroid gland, pancreas, kidney, and other organs. It is also known that the glandular syndrome progresses to the extraglandular syndrome in many cases and, though rarely, gives rise to malignant lymphoma.

20 As autoantibodies detected in Sjögren's syndrome, SS-A/Ro and SS-B/La are known. The corresponding antigens of these antibodies have already been identified but their specificity is low, with patients possessing autoantibodies to these antigens accounting for 40-60% of the total population of patients with

30 Sjögren's syndrome. Moreover, in many cases SS-A/Ro- or SS-B/La-positive patients have complications such as systemic lupus erythematosus and rheumatism [Journal of Clinical Investigation, 87, p68-76, 1991; and Nucleic Acids Research, 17, p2233-2244, 1989].

35 In view of the above findings, those antibodies or antigens are regarded as indicators of all autoimmune

diseases inclusive of Sjögren's syndrome. However, neither a specific autoantigen nor a specific autoantibody that could be a specific indicator of primary Sjögren's syndrome is not heretofore known.

5 α -Fodrin is one of subunits of fodrin which is a macromolecular actin-binding protein present immediately beneath the plasma membrane of cells. Fodrin forms high-order structures such as the fodrin network and is considered to be associated with the morphogenesis of cells and the migration of secretory
10 granules to the surface membrane.

It has recently been reported that in programmed cell deaths such as apoptosis, the full-length α -fodrin of 240K is restrictively cleaved by proteases to yield
15 α -fodrin protein fragments [Journal of Biological Chemistry, 270, p6425-6428, 1995]. Moreover, as the proteases causing such restricted cleavage of α -fodrin, trypsin, chymotrypsin, and calpain are known [Journal of Neuroscience, 8, p.2640-2651, 1988].

20 However, the relationship of this restricted cleavage of α -fodrin to Sjögren's syndrome remains to be known.

Therefore, if an autoantibody or autoantigen specific to Sjögren's syndrome, particularly primary
25 Sjögren's syndrome, is discovered, not only will the diagnosis of Sjögren's syndrome be facilitated and made more definite but the prophylaxis and therapy of Sjögren's syndrome, particularly primary Sjögren's syndrome, and, hence, prevention of progression of
30 primary to secondary syndrome will be made possible by establishing a tolerance to autoantigens prior to onset of the disease or after the onset.

Furthermore, detailed analyses for elucidation of the mechanisms of onset of Sjögren's syndrome will also
35 become feasible.

Summary of the Invention

In view of the above state of the art, the inventors of the present invention did much research and not only discovered autoantibodies which are specifically detected in primary Sjögren's syndrome but
5 also found that the corresponding autoantigens are α -fodrin and α -fodrin fragment proteins.

The inventors further found that Sjögren's syndrome can be prevented and cured by administering such an autoantigen.

10 The present invention, therefore, is directed to:

(1) A pharmaceutical composition comprising α -fodrin, a mutein thereof, a fragment thereof, or a salt thereof with a pharmaceutically acceptable carrier,

(2) A composition for preventing or treating
15 autoimmune disease comprising α -fodrin, a mutein thereof, a fragment thereof, or a salt thereof with a pharmaceutically acceptable carrier,

(3) A composition for preventing or treating Sjögren's syndrome comprising α -fodrin, a mutein thereof, a
20 fragment thereof, or a salt thereof with a pharmaceutically acceptable carrier,

(4) The composition described in the above item (3) wherein the molecular weight of said α -fodrin, a mutein thereof, or a fragment thereof is from about 2K to
25 about 240K,

(5) The composition described in the above item (3) wherein said α -fodrin, a mutein thereof, or a fragment thereof contains or comprises an amino acid sequence substantially shown by Arg-Gln-Lys-Leu-
30 Glu-Asp-Ser-Tyr-Arg-Phe-Gln-Phe-Phe-Gln-Arg-Asp-Ala-Glu-Glu-Leu,

(6) The composition described in the above item (5) wherein the molecular weight of said α -fodrin, a mutein thereof, or a fragment thereof is from about 100K to
35 about 140K,

(7) The composition described in the above item (3)

wherein said α -fodrin fragment is an α -fodrin fragment protein available upon proteolysis of α -fodrin with a protease,

- 5 (8) A diagnostic agent for autoimmune disease comprising α -fodrin, a mutein thereof, a fragment thereof, or a salt thereof,
- (9) A diagnostic agent for Sjögren's syndrome comprising α -fodrin, a mutein thereof, a fragment thereof, or a salt thereof,
- 10 (10) The diagnostic agent for Sjögren's syndrome described in the above item (9) wherein the molecular weight of α -fodrin, a mutein thereof, or a fragment thereof is from about 2K to about 240K,
- (11) The diagnostic agent for Sjögren's syndrome
- 15 described in the above item (9) wherein said α -fodrin, a mutein thereof, or a fragment thereof contains or comprises an amino acid sequence substantially shown by Arg-Gln-Lys-Leu-Glu-Asp-Ser-Tyr-Arg-Phe-Gln-Phe-Phe-Gln-Arg-Asp-Ala-Glu-Glu-Leu,
- 20 (12) The diagnostic agent for Sjögren's syndrome described in the above item (11) wherein the molecular weight of α -fodrin, a mutein thereof, or a fragment thereof is from about 100K to about 140K,
- (13) A method for detection or assay of an antibody
- 25 against α -fodrin, a mutein thereof, a fragment thereof, or a salt thereof, which comprises contacting α -fodrin, a mutein thereof, a fragment thereof, or a salt thereof with said antibody.
- (14) A method for preventing or treating autoimmune
- 30 disease which comprises administering to a patient a therapeutically effective amount of α -fodrin, a mutein thereof, a fragment thereof, or a salt thereof with a pharmaceutically acceptable carrier,
- (15) The method described in the above item (14),
- 35 wherein autoimmune disease is Sjögren's syndrome,
- (16) A method for diagnosing autoimmune disease which

comprises detecting or assaying autoantibody against α -fodrin, a mutein thereof, a fragment thereof, or a salt thereof, which comprises contacting α -fodrin, a mutein thereof, a fragment thereof, or a salt thereof with said autoantibody,

(17) The method described in the above item (16), wherein autoimmune disease is Sjögren's syndrome,

(18) Use of α -fodrin, a mutein thereof, a fragment thereof, or a salt thereof for the manufacture of a medicament for preventing or treating autoimmune disease,

(19) Use of α -fodrin, a mutein thereof, a fragment thereof, or a salt thereof for the manufacture of a medicament for preventing or treating Sjögren's syndrome,

(20) Use of α -fodrin, a mutein thereof, a fragment thereof, or a salt thereof for the manufacture of a medicament for diagnosing autoimmune disease, or

(21) Use of α -fodrin, a mutein thereof, a fragment thereof, or a salt thereof for the manufacture of a medicament for diagnosing Sjögren's syndrome.

Brief Description of the Drawings

Figure 1 shows electrophoregrams showing autoantigens in an NFS/sld mouse with Sjögren's syndrome as detected in its lacrimal gland (Lacrimal. gl) and salivary gland (Salivary. gl) extracts. In addition to autoantigens of 120K and 60K (indicated by arrowmarks), several other autoantigens, i.e. of 160K, 240K, etc., are observed.

Figure 2 shows electrophoregrams showing autoantigens in an athymic NFS/sld mouse at 4 and 12 weeks after operation at postnatal day 3. The legends "4 week", "12 week", and "BALB/c" indicate the serum from the athymic mouse after 4 weeks, the serum from the same mouse after 12 weeks, and the serum from a BALB/c mouse without Sjögren's syndrome, respectively.

Figure 3 shows gel filtration chromatogram of a murine salivary gland tissue homogenate. The recovered fraction is indicated by shading.

Figure 4 shows western blots of the fraction recovered after gel filtration chromatography of the murine salivary gland tissue homogenate as indicated by shading (Fig. 3) as well as the preceding and subsequent fractions using the serum from a mouse with Sjögren's-syndrome (Disease) or the serum from a mouse without Sjögren's syndrome (Normal).

Figure 5 shows ion-exchange chromatogram of the fraction recovered after gel filtration chromatography of the murine salivary gland tissue homogenate and the result of Western blotting using the recovered fraction (shaded area) and the serum from a mouse with Sjögren's syndrome.

Figure 6 shows western blots of the fusion protein (A) and α -fodrin fragment protein (B) obtained in Example 2 using the serum from a mouse with Sjögren's syndrome (Disease) or the serum from a mouse without the syndrome (Normal).

Figure 7 shows electrophoregrams showing the results of screening for autoantibodies to the α -fodrin fragment protein by the Western blotting method using the fusion protein obtained in Example 2 and the sera from patients with Sjögren's syndrome (Sjögren's syndrome), sera from patients with systemic lupus erythematosus (SLE), sera from patients with chronic arthritis (RA), or sera from healthy subjects (Normal).

Figure 8 shows electrophoregram (A) showing the calpain cleavage of murine α -fodrin protein as investigated using intact murine α -fodrin protein (α -fodrin) and murine calpain-treated α -fodrin protein (calpain treated) and the corresponding Western blots (B) using the serum from a mouse with Sjögren's syndrome.

Detailed Description of the Invention

α -Fodrin or a mutein thereof, a fragment thereof, or a salt thereof, which can be used in the present invention, includes but is not limited to human α -fodrin proteins, and there is no limitation on biological species or molecular weight provided that it is immunochemically equivalent to human α -fodrin protein itself or any fragment protein available on restricted cleavage of said α -fodrin protein by a protease or proteases.

The term "immunochemically equivalent" is used herein to mean the state of being biologically indistinguishable in antibody reactivity and antigenicity and, to cite a specific example, being recognizable by the same antibody or antiserum.

The above-mentioned α -fodrin, mutain, fragment protein, and salt may be those purified from organisms or those obtained by recombinant biotechnology. Particularly when they are produced by genetic engineering technology, there may be mutations, substitutions, insertions, additions or deletions of amino acids unless the immunochemical equivalence is lost. Moreover, side chains of the constituent amino acids of these proteins may be protected by suitable protective groups [e.g. C_{1-6} acyl groups such as formyl, acetyl, etc. (preferably C_{1-6} alkanoyl)] unless said immunochemical equivalence is lost.

To be more specific, the α -fodrin fragment protein that can be used in the present invention includes proteins available upon cleavage of human α -fodrin with proteases and is preferably one containing a partial sequence of human α -fodrin, particularly the amino acid sequence of SEQ ID NO:1 or an amino acid sequence immunochemically equivalent thereto.

The protease that can be used with advantage includes trypsin, chymotrypsin, and calpain (preferably

calpain).

In addition to the above proteins, α -fodrans derived from any tissues (e.g. brain) or cells of warm-blood animals (e.g. guinea pig, rat, mouse, rabbit, swine, sheep, cattle, monkey, etc.) or the proteins available upon proteolysis of the α -fodrans with said proteases. Preferred are proteins containing the amino acid sequence of human α -fodrin shown by SEQ ID NO:1 or an amino acid sequence immunochemically equivalent thereto and being immunochemically equivalent as the whole. The cleavage of α -fodrin protein with a protease can be achieved by allowing them to react in a buffer solution of pH from about 6 to about 8 at from about 10 to about 50°C (preferably from about 30 to about 45°C) for from about 30 minutes to about 5 hours.

Therefore, although the quantitative and qualitative factors such as amino acid composition of α -fodrin or its fragment protein may be diverse, its molecular weight is preferably from about 2K to about 240K and, for still better results, from about 100K to about 140K. Particularly preferred is one showing a molecular weight of 120K as determined by SDS-polyacrylamide gel electrophoresis.

The salt of α -fodrin and of an α -fodrin fragment protein in the context of the present invention may be any kind of salt but is preferably an acid addition salt and, in particular, a physiologically acceptable acid addition salt.

The preferred examples of such salt are salts with inorganic acids (e.g. hydrochloric acid, phosphoric acid, hydrobromic acid, sulfuric acid, etc.) or salts with organic acids (e.g. acetic acid, formic acid, propionic acid, fumaric acid, maleic acid, succinic acid, tartaric acid, citric acid, malic acid, oxalic acid, benzoic acid, methanesulfonic acid, benzenesulfonic acid, etc.).

The mutein of α -fodrin protein in the context of the present invention is a protein available upon mutation, substitution, insertion, addition and/or deletion of amino acids of α -fodrin protein barring
 5 loss of immunochemical equivalence.

The mutein more specifically includes a variety of proteins available upon mutation of the amino acid sequence of the parent protein, for example those available upon addition of amino acids, deletion of
 10 constituent amino acids, or substitution of different amino acids for constituent amino acids.

The addition of amino acids includes addition of at least one amino acid (preferably at most 5 amino acids).

15 The deletion of constituent amino acids includes deletion of at least one amino acid (preferably at most 5 amino acids) constituting α -fodrin.

The substitution of other amino acids includes substitution of at least one amino acid (preferably at
 20 most 5 amino acids) of α -fodrin.

The number of added amino acids is at least one but not limited unless the characteristics of α -fodrin, particularly immunological equivalence to the intact α -fodrin protein, are lost.

25 The number of constituent amino acids deleted in the deletion mutein of α -fodrin is at least one but there is no limitation on the number of deletions unless the characteristics of α -fodrin, particularly the immunological equivalence to α -fodrin protein, are
 30 lost.

The number of constituent amino acids of α -fodrin substituted in the substitution mutein is at least one but not limited unless the characteristics of α -fodrin, particularly immunological equivalence to the intact
 35 α -fodrin protein, are lost.

The constituent amino acids replaced in the

substitution muitein may be cysteine and/or other amino acids but preferably cysteine is replaced. The amino acids other than cysteine that may be replaced are aspartic acid, arginine, glycine, and valine, among
5 others.

When the constituent amino acid replaced is cysteine, the substitute amino acid is preferably a neutral amino acid, for instance. The neutral amino acid that can be used includes but is not limited to
10 glycine, valine, alanine, leucine, isoleucine, tyrosine, phenylalanine, histidine, tryptophan, serine, threonine, and methionine. Particularly preferred are serine and threonine.

Where the constituent amino acid to be replaced is
15 an amino acid other than cysteine, the substitute amino acid is selected from among those amino acids which are different from the amino acid to be replaced in hydrophilicity or hydrophobicity, or in electric charge. Specifically, when the amino acid to be replaced is
20 aspartic acid, for instance, the substitute amino acid may for example be asparagine, thereonine, valine, phenylalanine, or arginine, but asparagine or arginine is particularly preferred.

When the amino acid to be replaced is arginine,
25 the substitute amino acid may for example be glutamine, threonine, leucine, phenylalanine, or aspartic acid, although glutamine is particularly preferred.

When the amino acid to be replaced is glycine, the substitute amino acid may for example be threonine,
30 leucine, phenylalanine, serine, glutamic acid, or arginine, but threonine is particularly preferred.

When the amino acid to be replaced is serine, the substitute amino acid may for example be methionine, alanine, leucine, cysteine, glutamine, arginine, or
35 aspartic acid but methionine is particularly preferred.

When the amino acid to be replaced is valine, the

substitute amino acid may for example be serine, leucine, proline, glycine, lysine, or aspartic acid, but serine is particularly preferred.

5 The preferred amino acids to be replaced are aspartic acid, arginine, glycine, serine, valine, etc. and the preferred substitute amino acids are asparagine, glutamine, arginine, threonine, methionine, serine, and leucine.

10 In the substitution mutein, two or more substitutions may be concurrently involved. It is particularly preferable that 2 or 3 amino acids be substituted.

The above mutein may represent a combination of any two or three of the addition, deletion, and substitution described above. For the production of such a mutein, the method for site-directed mutagenesis is employed. This well-known method is described in Lather, R. F. and Lecoq, J. P., Genetic Engineering, p.31-50, Academic Press, 1983. The oligonucleotide-directed mutagenesis technique is described in Smith, M. and Gillam, S., Genetic Engineering, Principles and Methods, Vol. 3, p.1-32, Prenam Press, 1981.

The structural gene coding for the mutein can be produced typically by the following procedure.

(a) Hybridize a single-stranded DNA comprising the single-stranded DNA chain of the structural gene of α -fodrin with a mutagenic oligonucleotide primer (this primer must be complementary to the cysteine codon to be replaced or, depending on cases, a domain including the antisense triplet pairing with said codon.

25 However, mismatch with other amino acid encoding codons or, depending on cases, the antisense triplet is acceptable).

(b) Extend the primer with DNA polymerase to produce a mutagenic heteroduplex.

35 (c) Replicate this mutagenic heteroduplex.

Then, the phage DNA transporting the mutated gene

is isolated and introduced into a plasmid.

The plasmid thus obtained is used to transform a suitable host and the resulting transformant is grown in a medium so as to produce the desired mutein.

5 While the α -fodrin, α -fodrin fragment thereof, or a salt thereof can be produced in accordance with the methodology described above, they can also be produced by culturing a transformant containing a DNA fragment encoding the full-length α -fodrin or α -fodrin fragment
10 protein as described hereinafter.

Furthermore, they may also be produced by the method for peptide synthesis as described hereinafter.

The DNA sequence coding for the complete sequence of human α -fodrin protein for use in the present
15 invention is disclosed in Journal of Biological Chemistry, 265, p.4427-4433, 1990. Therefore, for the production of said α -fodrin or α -fodrin fragment protein, the DNA fragment can be obtained and either the whole or a portion be conveniently used.

20 The full length or part of the DNA can be cloned from the genomic DNA of a warm-blood animal (e.g. man etc.), the genome DNA library of a warm-blooded animal (e.g. man etc.), the cDNA of a warm-blooded animal (e.g. man etc.) tissue or cell, or the cDNA library of
25 a warm-blooded animal (e.g. man etc.) tissue or cell by the following procedure.

The vector for the library may be a bacteriophage, plasmid, cosmide, or phagimide.

Moreover, an mRNA fraction obtained from a tissue
30 or cell can be amplified by the known reverse transcriptase-polymerase chain reaction technique (RT-PCR method).

Furthermore, partial base sequences may respectively be chemically synthesized and condensed
35 together.

The detailed procedures are as follows.

As regards the method for cloning the full length or a fragment of the DNA coding for α -fodrin and α -fodrin fragment protein for use in the present invention, the necessary amplification can be achieved by the known polymerase chain reaction method (PCR method) using synthetic DNA primers having a partial base sequence of α -fodrin fragment protein or screened by hybridization of a DNA fragment inserted in a suitable vector to a DNA fragment coding for α -fodrin or α -fodrin fragment protein or a synthetic DNA-conjugate thereof. The hybridization can be carried out typically by the method described in Molecular Cloning, 2nd ed. (J. Sambrook et. al., Cold Spring Harbor Lab. Press, 1989). When a commercial library is used, the procedure described in the accompanying manual can be followed.

The cloned DNA fragment coding for α -fodrin and α -fodrin fragment protein can be used either as it is or, if desired, after digestion with a restriction enzyme or after addition of a linker. This DNA fragment may have TAG as the translation initiation codon at its 5'-end and TAA, TGA, or TAG as the translation termination codon at its 3'-end. The translation initiation codon and translation termination codon can also be added through suitable synthetic DNA adapters.

The expression vector for α -fodrin and α -fodrin fragment protein can be produced by, for example, (a) cloning the objective DNA fragment from the DNA encoding the α -fodrin or α -fodrin fragment protein of this invention and

(b) ligating this DNA fragment downstream of the promoter in a suitable expression vector.

The vector that can be used includes plasmids of the E. coli origin (e.g. pBR322, pBR325, pUC12, pUC13, etc.), plasmids of the B. subtilis origin (e.g. pUB110,

pTP5, PC194, etc.), plasmids of the yeast origin (e.g. pSH19, pSH15, etc.), λ phage and other bacteriophages, and animal viruses such as retrovirus, vaccinia virus, baculovirus, etc.

5 The promoter that can be used in the present invention can be any suitable promoter for the host used in gene expression.

 When the host to be transfected is a microorganism of the genus Escherichia, trp promoter, lac promoter, 10 recA promoter, λ PL promoter, lpp promoter, etc. can be used. When the host is a microorganism of the genus Bacillus, SP01 promoter, SP02 promoter, penP promoter, etc. can be employed. When the host is a yeast, PH05 promoter, PGK promoter, GAP promoter, ADH promoter, 15 etc. can be used with advantage.

 When the host is an animal cell, SV40-derived promoter, retrovirus promoter, metallothionein promoter, heat shock promoter, cytomegarovirus promoter, and SR α promoter, among others, can be 20 employed.

 In this connection, it is rewarding to use any of such promoters in conjunction with an enhancer compatible with the particular promoter.

 When the host is a microorganism of the genus 25 Escherichia, the alkaline phosphatase-signal sequence or OmpA-signal sequence, for instance, can be employed. When the host is a microorganism of the genus Bacillus, the α -amylase-signal sequence, subtilisin-signal sequence, or the like can be used. When the host is a 30 yeast, the mating factor α -signal sequence, invertase-signal sequence, or the like can be used. When the host is an animal cell, the insulin-signal sequence, α -interferon-signal sequence, antibody molecule-signal sequence, etc. can be utilized.

35 Where necessary, a signal sequence suited to the host can be added to the 5'-end of the DNA fragment

coding for the α -fodrin or α -fodrin fragment protein.

Moreover, where necessary, the sequence of a marker protein such as glutathione S-transferase or the like may be added to either the 5'-end or the 3'-end of the DNA fragment encoding α -fodrin or an α -fodrin fragment protein. This marker sequence is useful for the purification of α -fodrin protein [Gene, 67, p.31, 1988]. Moreover, the marker protein can be separated from α -fodrin or α -fodrin fragment protein by, for example, cleavage with a protease after optional purification [Gene, 67, p.31, 1988].

Using a vector containing the thus-constructed DNA fragment coding for α -fodrin or α -fodrin fragment protein, a transformant can be produced. As the host, a microorganism of the genus Escherichia or of the genus Bacillus, a yeast, an insect, or an animal cell can be employed.

The microorganism of the genus Escherichia that can be employed includes Escherichia coli K12•DH1 [Proc. Natl. Acad. Sci. U.S.A, 60, p.160, 1968], JM103 [Nucleic Acids Research, 9, p.309, 1981], JA221 [Journal of Molecular Biology, 120, p.517, 1978], HB101 [Journal of Molecular Biology, 41, p.459, 1969], and C600 [Genetics, 39, p.440, 1954], among other strains.

The microorganism of the genus Bacillus that can be employed includes Bacillus subtilis ML114 [Gene, 24, p.255, 1983] and 207-21 [Journal of Biochemistry, 95, p.87, 1984], among others.

The yeast that can be used includes Saccharomyces cerevisiae AH22, AH22R⁻, A87-11A, DXD-5D, and 20B-12, among others.

The insect that can be used includes silkworm larva [Maeda et al., Nature, 315, p.592, 1985].

The animal cell that can be used includes but is not limited to monkey cell line COS-7, Vero cell line,

Chinese hamster cell line CHO, DHFR gene-defected Chinese hamster cell line CHO (dhfr⁻CHO cell line), mouse L cell, mouse myeloma cell, and human FL cell line, among other cells.

- 5 Transfection of a microorganism of the genus Escherichia can be carried out typically by the methods described in Proc. Natl. Acad. Sci. USA, 69, p.2110 (1972) and Gene, 17, p.107 (1982).

- 10 Transfection of a microorganism of the genus Bacillus can be carried out typically by the methods described in Molecular & General Genetics, 168, p.111 (1979) and other literature.

- 15 Transfection of a yeast can be carried out typically by the method described in Proc, Natl, Acad, Sci, USA, 75, p.1929 (1978).

Transfection of an insect cell can be carried out typically by the method described in Bio/Technology, 6, p.47-55, (1988).

- 20 Transfection of an animal cell can be carried out typically by the method described in Virology, 52, p.456 (1973).

- 25 In this manner, a transformant produced by transfection with an expression vector containing a DNA fragment encoding α -fodrin or α -fodrin fragment protein can be obtained.

- 30 The culture medium that can be used for culture of a transformant obtained by using a microorganism of the genus Escherichia or Bacillus as the host is preferably a liquid medium containing sources of carbon and nitrogen, inorganic salts, and other components necessary for multiplication of the transformant.

- 35 The source of carbon that can be used includes glucose, dextrin, soluble starch, sucrose, etc. and the source of nitrogen includes varieties of inorganic or organic nitrogenous substances such as ammonium salts, nitrates, corn steep liquor, peptone, casein, meat

extract, soybean cake, and potato extract. Among the inorganic salt that can be used are calcium chloride, sodium dihydrogen phosphate, and magnesium chloride, to mention just a few.

5 Further, yeast extract, vitamins, growth factors, etc. can also be added.

The pH of the medium is preferably from about 5 to about 8.

10 The preferred culture medium that can be used for culture of a microorganism of the genus Escherichia includes M19 medium supplemented with glucose and casamino acids [Miller, Journal of Experiments in Molecular Genetics, p.431-433, Cold Spring Harbor Laboratory, New York, 1972]. To this medium may be
15 added a certain chemical, such as 3 β -indolylacrylic acid, where necessary, so as to allow the promoter to function with improved efficiency.

When the host is a microorganism of the genus Escherichia, the transformant is usually cultured at
20 from about 15 to about 43°C for about from 3 to about 24 hours, with aeration and agitation where necessary.

When the host is a microorganism of the genus Bacillus, the transformant is usually cultured at from
25 about 30 to about 40°C for from about 6 to about 24 hours, with aeration and agitation where necessary.

The medium that can be used for culture of a transformant constructed using a yeast as the host includes but is not limited to Burkholder's Minimal
30 Medium [Bostian, X. L. et. al., Proc. Natl. Acad. Sci USA, 77, p.4505 (1980)] and 0.5% casamino acids-supplemented SD Medium [Bitter, G. A. et al., Proc Natl. Acad. Sci. USA, 81, p.5330 (1984)]. The pH of the medium is preferably adjusted to form about 5 to
35 about 8. The transformant is cultured usually at from about 20 to about 35°C for from about 24 to about 72

hours, with aeration and agitation where necessary.

When the host is an insect cell, the culture medium that can be used for culture of the transformant includes Grace's Insect Medium (Grace, T. C. C.,
 5 Nature, 195, p.788 (1962) supplemented with suitable additives such as inactivated 10% bovine serum is used. The pH of the medium is preferably adjusted to from about 6.2 to about 6.4. The transformant is usually cultured at about 27°C for from about 3 to 5 days, with
 10 aeration and agitation where necessary.

For culture of the transformant constructed using an animal cell as the host, the culture medium that can be used includes but is not limited to MEM supplemented with from about 5 to about 20% fetal calf serum
 15 [Science, 122, p.501 (1952)], DMEM [Virology, 8, p.396 (1959)], RPMI1640 [The Journal of the American Medical Association, 199, p.519 (1967)], and 199 Medium [Proceeding of the Society for the Biological Medicine, 73, p.1 (1950)]. The pH of the medium is preferably
 20 from about 6 to about 8. The transformant is cultured generally at from about 30 to about 40°C for from about 15 to about 60 hours, with aeration and agitation where necessary.

Separation and purification of α -fodrin or α -fodrin fragment protein from the resulting culture
 25 broth can be typically carried out as follows.

For extration of the α -fodrin or α -fodrin fragment protein from grown bacterial or other cells, the cells are harvested by the known procedure and suspended in a
 30 suitable buffer solution. The suspended cells are disrupted by, for example, sonication, lysozyme treatment and/or freeze-thaw treatment and centrifuged or filtered to provide a crude extract of α -fodrin or α -fodrin fragment protein. The above-mentioned buffer
 35 may contain a protein denaturing agent such as urea or guanidine hydrochloride and/or a surfactant such as

Triton X-100 (the tradename of Sigma).

When α -fodrin or its fragment protein is secreted extracellularly, the grown bacterial and other cells are removed by a per se known procedure and the
5 supernatant is harvested.

The α -fodrin or α -fodrin fragment protein in the culture supernatant or extract can be purified by using per se known fractional purification techniques in a suitable combination.

10 Among such known fractional purification techniques are techniques utilizing a solubility differential, such as salting-out and solvent precipitation, techniques utilizing mainly a differential in molecular weight, such as dialysis,
15 ultrafiltration, gel permeation chromatography, and SDS-polyacrylamide gel electrophoresis, techniques utilizing a difference in electric charge, such as ion exchange chromatography, techniques depending on specific affinity, such as affinity chromatography, a
20 technique utilizing a difference in hydrophobicity, such as reversed phase high performance liquid chromatography, and techniques utilizing a differential in isoelectric point, such as electrofocusing.

The method for chemical synthesis of α -fodrin or
25 an α -fodrin fragment protein may be whichever of solid-phase synthesis and liquid-phase synthesis.

Thus, the objective peptide can be synthesized by condensing a peptide fragment or amino acid constituting the protein of the invention with the
30 remainder of the sequence and, where the product has protective groups, removing the protective groups.

The condensation and deprotection mentioned above can be carried out by, for example, the methods described in the following literature (1)-(5).

35 (1) M. Bodanszky and M. A. Ondetti, Peptide Synthesis, Interscience Publishers, New York (1966).

(2) Schroeder and Luebke, The Peptide, Academic Press. New York (1965).

(3) Izumiya, N. et al., Peptide Gosei no Kiso to Jikken (Fundamentals and Experiments in Peptide Synthesis), Maruzen, Ltd. (1975).

(4) Yatori, H. and Sakakibara, S., Seikagaku Jikken Koza 1 (Biochemical Experiment Series 1), Tanpakushitu no Kagaku (Protein Chemistry), p.205 (1977).

(5) Yatori, H. (ed.), Zoku Iyakuhin no Kaihatsu (Drug Development, Continued), vol. 14, Peptide Synthesis, Hirokawa Shoten.

After completion of the reaction, the protein of the present invention can be purified and isolated by using such techniques as solvent extraction, distillation, column chromatography, liquid chromatography, recrystallization, etc. in a suitable combination.

When the protein synthesized by the above method is a free peptide, it can be converted to a suitable salt by the known procedure. When a salt is obtained, it can be converted to the free peptide by the known procedure.

Thus, when the product α -fodrin, mutein, or fragment protein is a free peptide, it can be converted to a salt by a per se known procedure or a suitable procedure analogous therewith and when the product is a salt, it can be converted to the free peptide by the per se known procedure or a suitable procedure analogous therewith.

It is also possible to modify or trim the α -fodrin or α -fodrin fragment protein obtained by recombinant technology by causing a suitable protein-modifying enzyme to act on it either before or after purification.

The protein-modifying enzyme that can be used includes but is not limited to trypsin, chymotrypsin,

arginyl end peptidase, protein kinase, and glucosidase.

The α -fodrin, mutein, fragment protein, or salt according to the present invention can be used in the form of a medicinal composition as a prophylactic and therapeutic drug for autoimmune diseases such as systemic lupus erythematosus, rheumatism, Sjögren's syndrome, etc., particularly as a drug for the prevention and treatment of Sjögren's syndrome.

Specifically, by administering α -fodrin or a mutein thereof, a fragment protein thereof, or a salt thereof, inflammations associated with autoimmune diseases, particularly inflammations of the lacrimal and salivary glands in Sjögren's syndrome can be treated or prevented.

If desired, the α -fodrin, mutein, fragment protein, or salt of the invention can be administered in combination with other known immunosuppressants and/or antiinflammatory agents in a suitable regimen for the prevention and treatment of autoimmune diseases, particularly the prevention and treatment of Sjögren's syndrome.

For application as a medicine, the α -fodrin, mutein, fragment protein, or salt of the present invention can be administered either as it is in bulk form or in a dosage form containing it in combination with a pharmaceutically acceptable carrier, excipient or diluent (e.g. as an injection, tablets, capsules, a liquid, an ointment, or a suppository etc.) to warm-blooded animals (e.g. man etc.) safely by the oral or other route.

Injections can be prepared by using physiological saline or an aqueous solution of glucose or the like in the routine manner.

Tablets, capsules, and other dosage forms can also be manufactured by the established corresponding pharmaceutical procedures.

As a medicine, the α -fodrin, mutein, fragment protein, or salt of the present invention is administered to warm-blooded animals in the doses selected from the daily dose range of from about 1 ng to about 100 μ g/kg, preferably from about 10 ng to about 10 μ g/kg, taking the route of administration, clinical symptoms, and other factors into consideration.

The α -fodrin, mutein, fragment protein, or salt of the present invention can be used for the diagnosis of autoimmune diseases inclusive of Sjögren's syndrome.

Specifically, by detecting and assaying autoantibodies to the α -fodrin or α -fodrin fragment protein in the blood of a warm-blooded animal, the diagnosis, disease staging, or prediction of onset of autoimmune disease, particularly Sjögren's syndrome can be successfully accomplished.

The detection and assay of autoantibodies to α -fodrin and α -fodrin fragment protein can be made typically by the following method.

The α -fodrin, mutein, fragment protein, or salt (hereinafter referred to collectively as antigen protein) is coupled to a matrix such as cellulose beads in the routine manner. Then, the sample to be assayed is added and allowed to react at a given temperature (from about 4°C to about 40°C) for a given time.

After this reaction mixture is washed thoroughly, an antibody specifically binding to antibodies of the same species as that of the sample and labeled with a fluorescent substance, a chromogenic substance, an enzyme, or a radioisotope is added and allowed to react at a given temperature (from about 4°C to about 40°C) for a given time.

The reaction mixture is then washed thoroughly and, where necessary, a substrate for the enzyme is added and allowed to react at a given temperature (from

about 4°C to about 40°C) for a given time. Then, the optical density, intensity of fluorescence, or scincillation count of the reaction product is determined.

5 The matrix that can be used for supporting the antigen protein in the above assay method includes gel beads [e.g. agarose gel (e.g. Sepharose 4B, Sepharose 6B (the trademark of Pharmacia Fine Chemicals (Sweden)) etc.), dextran gel (e.g. Sephadex G-75, Sephadex G-100, 10 Sephadex G-200 (the trademark of Pharmacia Fine Chemicals (Sweden)) etc.), polyacrylamide gel (e.g. Bio-Gel P-30, Bio-Gel P-60, Bio-Gel P-100 (the trademark of Bio-Rad Laboratories (U.S.A.)) etc.)], cellulose beads [e.g. Avicel (the trademark of Asahi 15 Chemical Industry Co. Ltd.), ion exchange cellulose (e.g. diethylaminoethylcellulose, carboxymethyl-cellulose, etc.) etc.], physical adsorbents [e.g. glass (glass beads, glass rods, aminoalkyl glass beads, aminoalkyl glass rods, etc.), silicone flakes, styrenic 20 resin (e.g. polystyrene beads, polystyrene particles, etc.), immunoassay plates (e.g. the product of Nunc (Denmark)) etc.], ion exchange resin [e.g. weakly acidic cation exchange resin (e.g. Amberlite IRC-5 (the trademark of Rohm & Haas Company (U.S.A.)), Zeo-Karb 25 226 (the trademark of Permutit (West Germany)), and weakly basic anion exchange resin (e.g. Amberlite IR-4B, Dowex 3 (the trademark of Dow Chemical (U.S.A.)) etc.) etc.], among others.

30 The antigen protein can be immobilized on the matrix by the conventional procedures. For example, the cyanogen bromide method and glutaraldehyde method described in Metabolism, 8, p.696 (1971) can be mentioned. As a more expedient procedure, the antigen protein can be physically adsorbed on the matrix.

35 The label for the antibody-label conjugate (labeled antibody) can for example be a radioisotope,

an enzyme, a fluorescent substance, or a chromogen but the use of an enzyme is preferred.

The enzyme is preferably a stable enzyme with high specific activity, such as peroxidase, alkaline phosphatase, β -D-glactosidase, glucose oxidase, etc.,
5 although peroxidase is most preferred.

The peroxidase may be of any origin or type, thus including the species available from horseradish, pineapple, fig, sweet potato, kidney bean, and corn,
10 among other sources. Particularly preferred is horseradish peroxidase (HRP) extracted from horseradish.

In conjugating the antibody with such a peroxidase, it is convenient to use a maleimidated
15 peroxidase so as to utilize the thiol group of the antibody molecule Fab'. As a method for maleimiding a peroxidase, the maleimide group can be introduced via the amino group of the peroxidase. For this purpose, the N-succinimidyl derivative of a maleimidocarboxylic
20 acid can be employed and N-(γ -maleimidobutyryloxy)-succinimide (sometimes referred to briefly as GMBS) is used with advantage.

Thus, a certain intervening group may exist between the maleimide group and the peroxidase. To
25 cause GMBS to react with peroxidase, the two reactants are incubated in a buffer of pH from about 6 to about 8 at a temperature of from about 10 to about 50°C for from about 10 minutes to about 24 hours. The buffer mentioned above may for example be 0.1 M phosphate
30 buffer with a pH value of about 7.0.

The thus-maleimidated peroxidase can be purified by, for example, gel filtration chromatography.

The matrix that can be used for said gel filtration chromatography includes Sephadex G-25 [the
35 trademark of Pharmacia Fine Chemicals (Sweden)] and Bio-Gel P-2 [the trademark of Bio-Rad Laboratories

(U.S.A.)], among others. The reaction between antibody and maleimidated peroxidase can be carried out in a buffer at a temperature of from about 0°C to about 40°C for from about 1 to about 48 hours. The buffer may for example be 0.1 M phosphate buffer containing 5 mM sodium ethylenediaminetetraacetate (pH 6.0).

The peroxidase-labeled antibody thus obtained can be purified typically by gel filtration chromatography.

The support that can be used for said gel filtration chromatography includes Sephadex G-25 [the trademark of Pharmacia Fine Chemicals (Sweden)] and Bio-Gel P-2 [the trademark of Bio-Rad Laboratories (U.S.A.)], among others.

As an alternative, a thiol group may be introduced into peroxidase, which can then be reacted with the maleimidated antibody molecule.

Coupling of an enzyme other than peroxidase with the antibody can also be carried out in a similar manner and any of the glutaraldehyde method, periodic acid method, and water-soluble carbodiimide method, for instance, can be utilized.

The assay method according to the present invention is now described in detail, taking the use of peroxidase as the label as an example, although the label that can be used is not limited to peroxidase.

In a first place,

(1) A sample to be assayed is added to the antigen protein immobilized on a matrix to conduct an antigen-antibody reaction. Then, the above-prepared conjugate of peroxidase with an antibody binding to the antibodies of the same species as that of the sample (enzyme-labeled antibody) is added and reacted.

(2) To the reaction product obtained in (1), a substrate for peroxidase is added and the optical density or intensity of fluorescence of the reaction product is measured to calculate the enzymatic activity

of the reaction product.

(3) The above procedures (1) and (2) are followed using standard solutions of the anti- α -fodrin or α -fodrin fragment protein antibody occurring in the serum from a patient with Sjögren's syndrome beforehand to construct a standard curve correlating the amount of the anti- α -fodrin or α -fodrin fragment protein antibody with the optical density or intensity of fluorescence.

(4) Using the serum from a patient with Sjögren's syndrome or a risk for the syndrome, the above procedures (1) and (2) are repeated and the measured optical density or intensity of fluorescence are fitted to the above standard curve to find the amount of the patient's anti- α -fodrin or α -fodrin fragment protein antibody.

Similarly, the diagnosis of Sjögren's syndrome can be made in accordance with a pre-constructed correlogram between the amount of anti- α -fodrin or α -fodrin fragment protein antibody and the probability of onset of Sjögren's syndrome.

Where, in this specification and the accompanying drawings, any base, amino acid, etc. are designated by abbreviations, the abbreviations recommended by IUPAC-IUB Commission on Biochemical Nomenclature or those used commonly in the art are employed. Some examples are listed below. For any amino acid that may exist as optically active forms, the L-compound is meant unless otherwise indicated.

DNA : deoxyribonucleic acid
 cDNA : complementary deoxyribonucleic acid
 mRNA : messenger ribonucleic acid
 SDS : sodium dodecyl sulfate
 EDTA : ethylenediaminetetraacetic acid
 DEAE : diethylaminoethyl
 Arg : arginine
 Gln : glutamine

Lys : lysine
 Leu : leucine
 Glu : glutamic acid
 Asp : aspartic acid
 5 Ser : serine
 Tyr : tyrosine
 Phe : phenylalanine
 Ala : alanine

The SEQ ID NO mentiond under the title of Sequence
 10 Listing in this specification represents the following
 sequence.

SEQ ID NO:1

represents the amino acid sequence from the N-
 terminus through the 20-position of the autoantigen
 15 protein of 120K obtained from an NFS/sld mouse as shown
 in Example 1.

Examples

The following examples are intended to describe
 the present invention in further detail and should by
 20 no means be construed as defining the scope of the
 invention.

Example 1 Characterization of the autoantibodies and
 autoantigens in a Sjögren's syndrome model NFS/sld
 mouse

25 It is known that when thymectomized on day 3 after
 birth, NFS/sld mice develop Sjögren's syndrome in 4-20
 weeks after operation. Moreover, the symptoms observed
 then are known to well reflect the manifestations of
 primary Sjögren's syndrome [Hayashi, Y. and Haji, N.,
 30 Clinical Immunology, 27, p.488-492 (1995), Hayashi, Y.,
 Japanese Journal of Clinics, 53, 10, p.2383-2388
 (1995), or Hashima et al., Journal of Immunology, 153,
 p.2769 (1994)].

NFS/sld mice were thymectomized on day 3 after
 35 birth and the serum was harvested after 4 weeks and 12
 weeks. Using this serum and the serum obtained from

BALB/c mice without Sjögren's syndrome, the presence of specific autoantibodies to Sjögren's syndrome was investigated.

5 Mouse salivary gland and lacrimal gland tissues
were respectively homogenized in a Polytron homogenizer
and suspended in 20 mM Tris-HCl (pH 7.2) containing
0.15 M sodium chloride, 5 mM benzamidine HCl, 2 mM
diisopropyl fluorophosphate, 2 mM N-ethylmaleinimide, 2
mM phenylmethanesulfonyl fluoride, and 2 mM EDTA. The
10 suspension was centrifuged at 20000 x g for 30 minutes
and the supernatant was subjected to SDS-polyacrylamide
gel electrophoresis, transferred to a membrane filter,
and reacted with the mouse serum. To this was added
alkaline phosphatase-goat anti-mouse IgG antibody
15 conjugate for a color reaction utilizing phosphatase
activity. In this manner, autoantigens of 60K, 120K,
160K, and 240K were detected in the NFS/sld mouse
salivary and lacrimal gland tissue homogenates (Fig.
1).

20 At the same time, the presence of an
autoantibodies recognizing the autoantigen of 120K was
detected in the serum from NFS/sld mice which developed
Sjögren's syndrome 12 weeks after thymectomy (Fig. 2).

25 Then, for characterization of the 120K antigen
protein reacting most intensely with the
autoantibodies, the centrifugal supernatant of the
mouse salivary gland homogenate was applied to Superose
12HR column (Pharmacia) for gel permeation
chromatography using 20 mM Tris-HCl (pH 7.2) containing
30 1 M sodium chloride and 2 mM EDTA (Fig. 3). The
fraction containing the 120K molecule reacting with
NFS/sld mouse antiserum was recovered and subjected to
ion exchange chromatography (Fig. 4).

35 The recovered fraction was dialyzed against 20 mM
Tris-HCl (pH 7.2) containing 0.15 M sodium chloride and
2 mM EDTA and, then, applied to a DEAE-Cosmogel column

(Nakarai Chemical). Elution was carried out on a gradient of 0.15 M - 0.75 M sodium chloride (Fig. 5).

The fraction containing the 120K molecule reacting with NFS/sld mouse antiserum was recovered,
 5 refracted by 10% SDS-polyacrylamide electrophoresis, and transferred to a membrane filter. The clone corresponding to 120K was isolated and analyzed for amino acid sequence using Applied Biosystems 477A protein sequencer. As a result, the
 10 amino acid sequence from the N-terminus through 20-position of the above 120K autoantigen protein was in agreement with the known N-terminal sequence from 37-through 56-positions of human α -fodrin.

15 Example 2 Production of human α -fodrin fragment protein

The DNA coding for human α -fodrin protein is known [Journal of Biological Chemistry, 265, p.4427-4433 (1990)].

20 A human α -fodrin fragment protein was produced using JS-1 containing a DNA fragment coding for the N-terminal region of the above DNA sequence.

The JS-1 DNA fragment (1-1784 bp) was completely digested with the restriction enzyme EcoRI and ligated to the EcoRI site of E. coli pGEK-2T vector
 25 (Pharmacia). According to the manufacturer's instructions for this vector, the vector was introduced into E. coli and the glutathione S-transferase- α -fodrin fragment protein fusion protein was harvested. According to the instructions, this fusion protein was
 30 digested with thrombin and the α -fodrin fragment protein was isolated and purified independently of glutathione S-transferase.

Using the above fusion protein and said α -fodrin fragment protein, their reactivity with sera from
 35 NFS/sld mice presenting with Sjögren's syndrome and NFS/sld mice not presenting with Sjögren's syndrome was

investigated by Western blotting as in Example 1 (Fig. 6).

It was found that both the fusion protein and the α -fodrin fragment protein react with serum from the model mouse.

It was also found that since glutathione S-transferase does not react with sera from warm-blooded animals, the above fusion protein can be used without further processing for the purposes of the present invention.

Example 3 The presence of α -fodrin fragment protein autoantigens in humans with Sjögren's syndrome

The fusion protein obtained in Example 2 was subjected to SDS-polyacrylamide gel electrophoresis and transferred to a filter membrane for immunoblotting. Then, using sera from patients with Sjögren's syndrome, sera from patients with systemic lupus erythematosus, sera from patients with chronic arthritis, or sera from healthy volunteers, autoantibodies to α -fodrin fragment protein were explored by the routine Western blotting technique (Fig. 7).

As a result, no autoantibody to the α -fodrin fragment protein was detected in the sera from patients with systemic lupus erythematosus or those with chronic arthritis. On the other hand, autoantibodies to the α -fodrin fragment protein of the present invention were found in sera from patients with Sjögren's syndrome.

It was, thus, clear that expression of autoantibodies to the α -fodrin fragment protein is specific to Sjögren's syndrome.

Example 4 The inhibitory effect of human α -fodrin fragment protein on the onset of Sjögren's syndrome

The inhibitory effect of the fusion protein obtained in Example 2 on the onset of Sjögren's syndrome was investigated.

Athymic NFS/sld mice, 3 weeks after operation at

postnatal day 3, were intravenously dosed with a saline solution (25 µg/0.1ml) of the fusion protein obtained in Example 2 or, as control, of either albumin-lysozyme or glutathione S-transferase. The animals were

5 autopsied 8 weeks after administration and the severity of lesion was assessed by the method of White and Casarett using pathological indicators [Journal of Immunology, 112, p.178 (1974)]. The results are shown in Table 1.

10 In the group dosed with the α-fodrin fragment protein-glutathione S-transferase fusion protein, significant alleviations of lesions were found in all the submandibular gland, parotid gland and lacrimal gland tissues. In other organs, no pathological change

15 was found.
[Table 1]

Treatment	No. of mice examined	Grade of lesion (mean±S.D)		
		Parotid gland	Submandibular gland	Lacrimal gland
Untreated	n=6	2.52±0.16*	2.28±0.18*	2.17±0.26*
α-fodrin	n=7	0.28±0.49*	0.14±0.38*	0.28±0.49*
Albumin	n=5	2.55±0.19	2.23±0.14	2.01±0.32
Lysozyme	n=5	2.48±0.13	2.34±0.18	2.18±0.29
Control	n=5	2.58±0.22	2.38±0.28	2.22±0.34

20 *: Significantly different at p<0.01,
Mann-Whitney's U test

Table 1 shows the relative efficacies for the symptomatic improvement of Sjögren's syndrome of no treatment (untreated), glutathione S-transferase-α-fodrin fragment protein fusion protein (α-fodrin),

35 albumin, lysozyme, and glutathione S-transferase.
Example 5 Calpain-proteolysis of α-fodrin protein

A mouse α-fodrin protein purified by the per se known method, 3 µg, was suspended in 50 mg imidazole hydrochloride (pH 7.2)-5 mM cysteine-1 mM calcium

40 chloride and treated with 0.5 U of murine calpain

(Sigma) at 37°C for 60 minutes. The lysate was separated by SDS-polyacrylamide gel electrophoresis and Western blotted using antiserum from a mouse with Sjögren's syndrome (Fig. 8).

5 The antibodies to the α -fodrin or α -fodrin fragment protein of the present invention are detected in coincidence with the onset of Sjögren's syndrome which is an autoimmune disease. Therefore, the antigen protein of the present invention is of value as a
10 diagnostic agent for autoimmune disease, particularly Sjögren's syndrome. Furthermore, since the antigen protein of the present invention remarkably inhibits the onset of illness when administered to a murine model of Sjögren's syndrome, it is apparent that the
15 protein is of value as a prophylactic and therapeutic agent for autoimmune disease, particularly Sjögren's syndrome.

[Sequence table]

SEQ ID NO: 1

20 Length: 20

Type: amino acid

Topology: linear

Sequence type: peptide

Arg Gln Lys Leu Glu Asp Ser Tyr Arg Phe Gln Phe Phe Gln

25 Arg Asp Ala Glu Glu Leu

(i) APPLICANT: Hayashi, Yoshio
Sugino, Hiromu

(ii) TITLE OF INVENTION: COMPOSITION CONTAINING α -FODRIN OR α -FODRIN
FRAGMENT PROTEIN

(iii) NUMBER OF SEQUENCES: 1

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: Dike, Bronstein, Roberts & Cushman, LLP
(B) STREET: 130 Water Street
(C) CITY: Boston
(D) STATE: MA
(E) COUNTRY: USA
(F) ZIP: 02109

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Diskette, 3.5 inch, 1.44 MB
(B) COMPUTER: IBM PC
(C) OPERATING SYSTEM: Dos 5.0
(D) SOFTWARE: WordPerfect 5.1

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(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: David S. Resnick, Esq.
(B) REGISTRATION NUMBER: 34,235
(C) REFERENCE/DOCKET NUMBER: 46,910

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: 617-523-3400
(B) TELEFAX: 617-523-6440
(C) TELEX:

(1) INFORMATION FOR SEQ ID NO: 1

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 20
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULAR TYPE: peptide

(iii) SEQUENCE DESCRIPTION: SEQ ID NO: 1

Arg Gln Lys Leu Glu Asp Ser Tyr Arg Phe Gln Phe Phe Gln Arg
1 5 10 15
Asp Ala Glu Glu Leu
20

What is claimed is:

1. A pharmaceutical composition comprising α -fodrin, a mutein thereof, a fragment thereof, or a salt thereof with a pharmaceutically acceptable carrier.

2. A composition for preventing or treating autoimmune disease comprising α -fodrin, a mutein thereof, a fragment thereof, or a salt thereof with a pharmaceutically acceptable carrier.

3. A composition for preventing or treating Sjögren's syndrome comprising α -fodrin, a mutein thereof, a fragment thereof, or a salt thereof with a pharmaceutically acceptable carrier.

4. The composition of claim 3 wherein the molecular weight of said α -fodrin, a mutein thereof, or a fragment thereof is from about 2K to about 240K.

5. The composition of claim 3 wherein said α -fodrin, a mutein thereof, or a fragment thereof contains or comprises an amino acid sequence substantially shown by Arg-Gln-Lys-Leu-Glu-Asp-Ser-Tyr-Arg-Phe-Gln-Phe-Phe-Gln-Arg-Asp-Ala-Glu-Glu-Leu.

6. The composition of claim 5 wherein the molecular weight of said α -fodrin, a mutein thereof, or a fragment thereof is from about 100K to about 140K.

7. The composition of claim 3 wherein said α -fodrin fragment is an α -fodrin fragment protein available upon proteolysis of α -fodrin with a protease.

8. A diagnostic agent for autoimmune disease comprising α -fodrin, a mutein thereof, a fragment thereof, or a salt thereof.

9. A diagnostic agent for Sjögren's syndrome comprising α -fodrin, a mutein thereof, a fragment protein thereof, or a salt thereof.

10. The diagnostic agent for Sjögren's syndrome of claim 9 wherein the molecular weight of α -fodrin, a mutein thereof, or a fragment thereof is from about 2K to about 240K.

11. The diagnostic agent for Sjögren's syndrome of claim 9 wherein said α -fodrin, a mutein thereof, or a fragment thereof contains or comprises an amino acid sequence substantially shown by Arg-Gln-Lys-Leu-Glu-Asp-Ser-Tyr-Arg-Phe-Gln-Phe-Phe-Gln-Arg-Asp-Ala-Glu-Glu-Leu.
12. The diagnostic agent for Sjögren's syndrome of claim 11 wherein the molecular weight of α -fodrin, a mutein thereof, or a fragment thereof is from about 100K to about 140K.
13. A method for detection or assay of an antibody against α -fodrin, a mutein thereof, a fragment thereof, or a salt thereof, which comprises contacting α -fodrin, a mutein thereof, a fragment thereof, or a salt thereof with said antibody.
14. A method for preventing or treating autoimmune disease which comprises administering to a patient a therapeutically effective amount of α -fodrin, a mutein thereof, a fragment thereof, or a salt thereof with a pharmaceutically acceptable carrier.
15. The method of claim 14, wherein autoimmune disease is Sjögren's syndrome.
16. The method of claim 15, wherein the molecular weight of said α -fodrin, a mutein thereof, or a fragment thereof is from about 2K to about 240K.
17. The method of claim 15, wherein said α -fodrin, a mutein thereof, or a fragment thereof contains or comprises an amino acid sequence substantially shown by Arg-Gln-Lys-Leu-Glu-Asp-Ser-Tyr-Arg-Phe-Gln-Phe-Phe-Gln-Arg-Asp-Ala-Glu-Glu-Leu.
18. The method of claim 17, wherein the molecular weight of said α -fodrin, a mutein thereof, or a fragment thereof is from about 100K to about 140K.
19. The method of claim 18, wherein said α -fodrin fragment is an α -fodrin fragment protein available upon proteolysis of α -fodrin with a protease.

20. A method for diagnosing autoimmune disease which comprises detecting or assaying an autoantibody against α -fodrin, a mutein thereof, a fragment thereof, or a salt thereof, which comprises contacting α -fodrin, a mutein thereof, a fragment thereof, or a salt thereof with said antibody.

21. A method for diagnosing Sjögren's syndrome which comprises detecting or assaying an autoantibody against α -fodrin, a mutein thereof, a fragment thereof, or a salt thereof, which comprises contacting α -fodrin, a mutein thereof, a fragment thereof, or a salt thereof with said antibody.

22. The method of claim 21, wherein the molecular weight of α -fodrin, a mutein thereof, or a fragment thereof is from about 2K to about 240K.

23. The method of claim 21, wherein said α -fodrin, a mutein thereof, or a fragment thereof contains or comprises an amino acid sequence substantially shown by Arg-Gln-Lys-Leu-Glu-Asp-Ser-Tyr-Arg-Phe-Gln-Phe-Phe-Gln-Arg-Asp-Ala-Glu-Glu-Leu.

24. The method of claim 23, wherein the molecular weight of α -fodrin, a mutein thereof, or a fragment thereof is from about 100K to about 140K.

Abstract

Screening of the autoantibodies and autoantigens which are specific to Sjögren's syndrome, particularly primary Sjögren's syndrome, and provision of a prophylactic and therapeutic drug for Sjögren's syndrome, particularly primary Sjögren's syndrome, and a highly sensitive diagnostic agent for Sjögren's syndrome. The α -fodrin or α -fodrin fragment of the invention is an antigen to the autoantibodies specifically found in Sjögren's syndrome and this autoantigen finds application as a prophylactic and therapeutic drug for autoimmune diseases, particularly Sjögren's syndrome, or a diagnostic agent for such diseases.

Figure 1

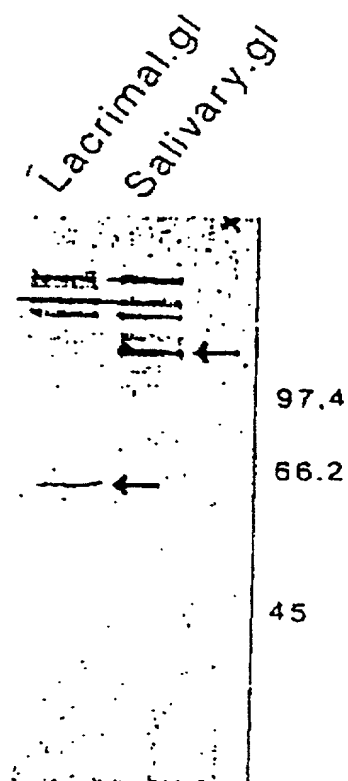


Figure 2

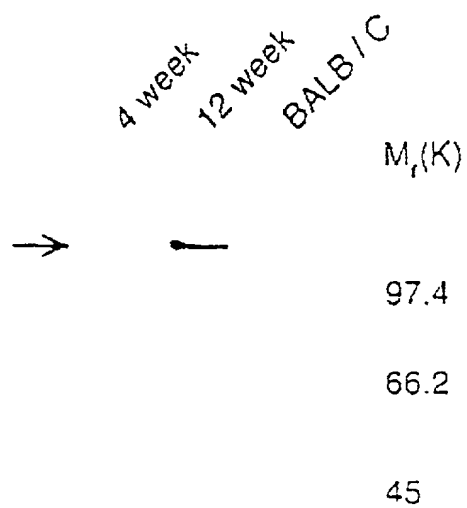


Figure 3

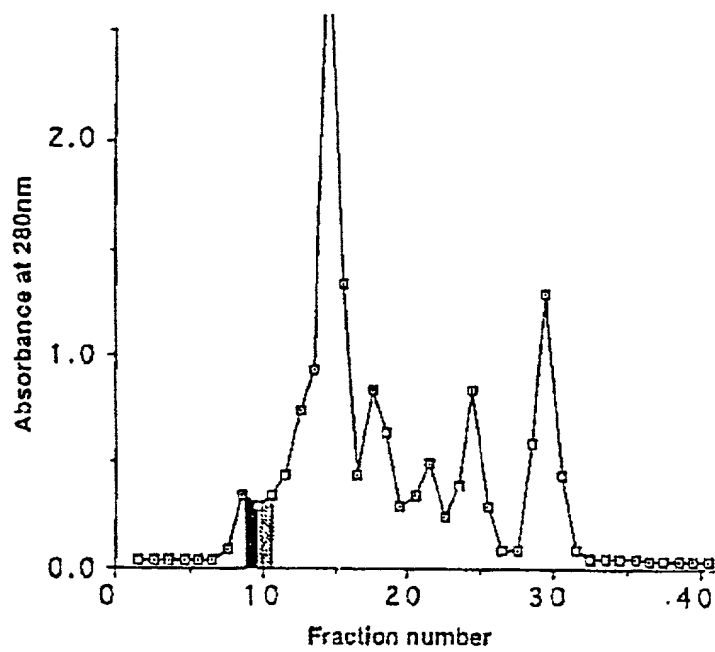


Figure 4

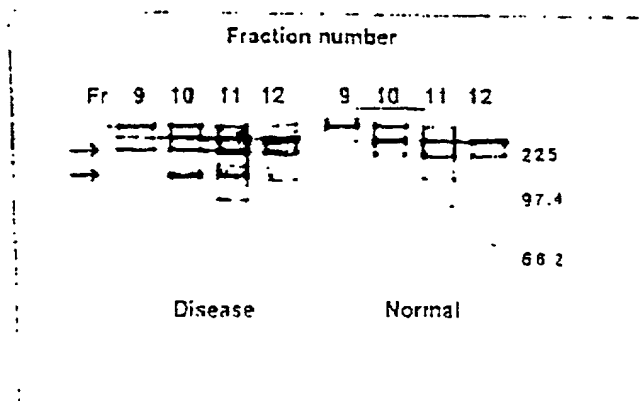


Figure 5

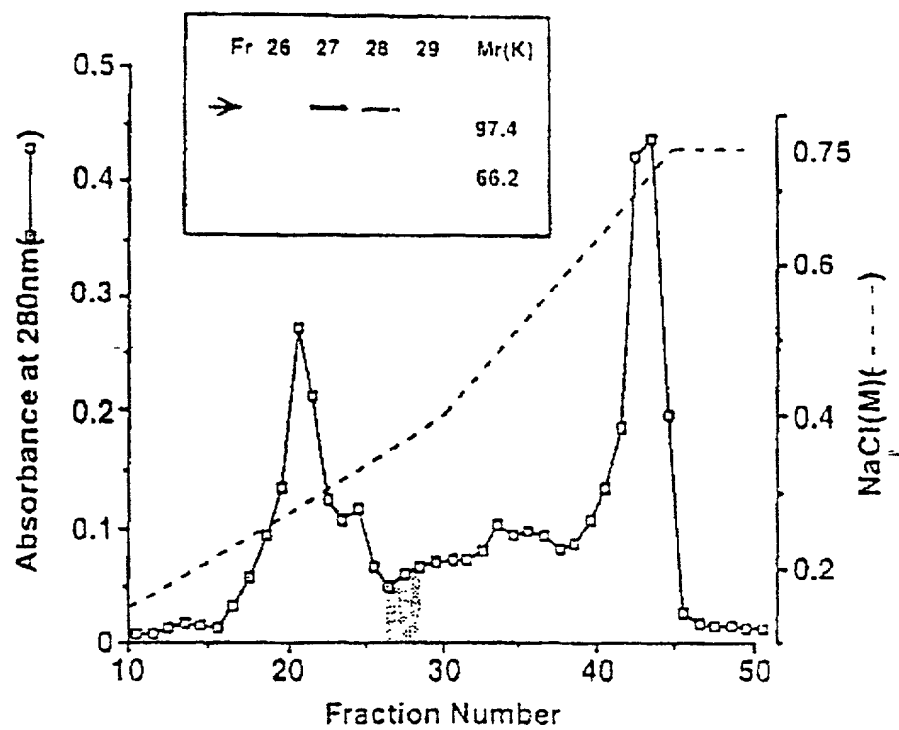


Figure 6

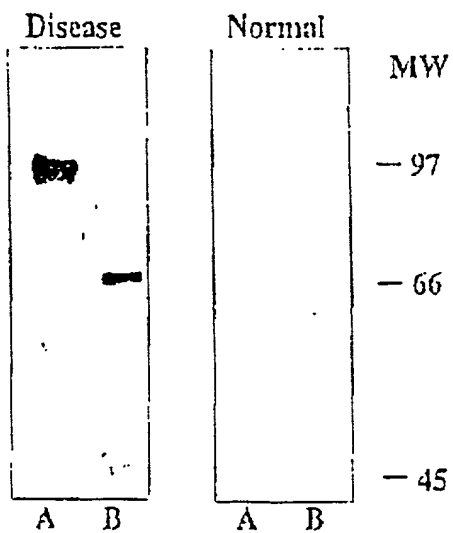


Figure 7

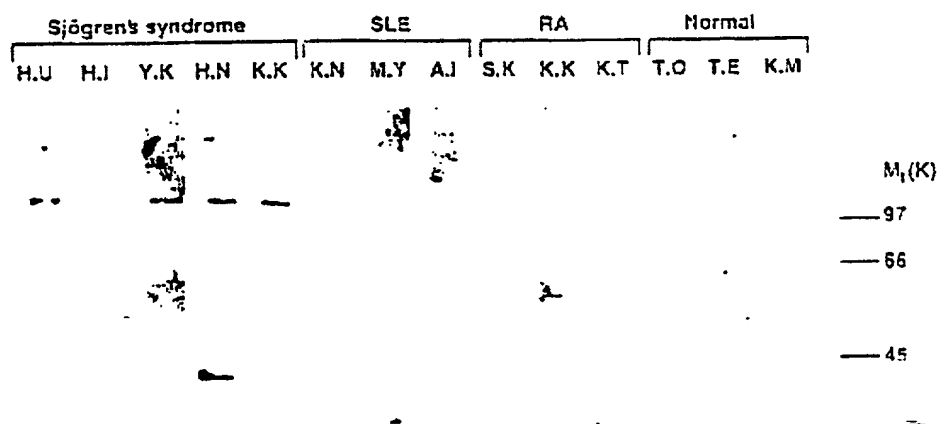
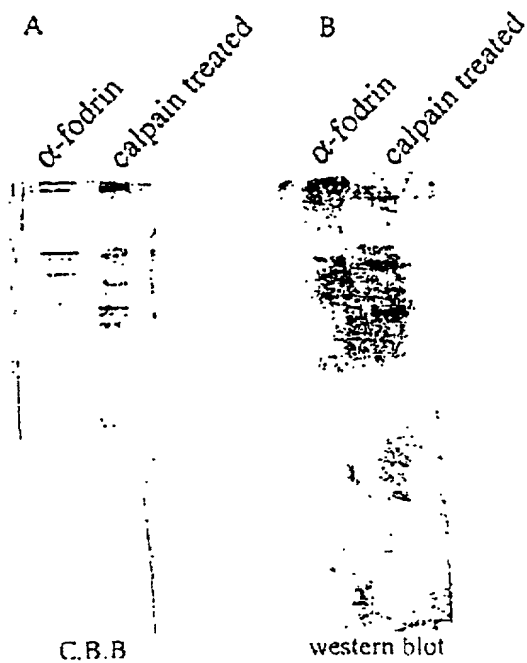


Figure 8



DIKE, BRONSTEIN, ROBERTS & CUSHMAN
130 Water Street
Boston, Massachusetts 02109

DECLARATION AND POWER OF ATTORNEY
Original Application

As a below named inventor, I hereby declare that: My residence, post office address and citizenship are as stated below next to my name. I believe I am the original, first and sole inventor (if only one name is listed at 201) below or an original, first and joint inventor (if plural names are listed at 201-208 below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

Composition Containing α -fodrin or α -fodrin Fragment Protein

which is described and claimed in: ☐ the attached specification or ☒ the specification in application Serial No. 08/736,434
filed October 24, 1996 And was amended on _____
if applicable

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above. I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, §1.56(a). I hereby claim foreign priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed.

FOREIGN APPLICATION(S), IF ANY, FILED WITHIN 12 MONTHS PRIOR TO THE FILING DATE OF THIS APPLICATION				
COUNTRY	APPLICATION	DATE OF FILING (day, month, year)	PRIORITY CLAIMED UNDER 35 U.S.C. §119	
JAPAN	100765/1996	23/4/96	Yes	
ALL FOREIGN APPLICATIONS, IF ANY, FILED MORE THAN 12 MONTHS PRIOR TO THE FILING DATE OF THIS APPLICATION				

I hereby claim the benefit under Title 35, United States code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112. I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

(Application Serial No.)	(Filing Date)	(Status) (Patented, pending, abandoned)

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) with full powers of association, substitution and revocation to prosecute this application and transact all business in the Patent and Trademark Office connected therewith. *(list name and registration number)*

Sewall P. Bronstein (Reg. No. 16,919)	Ronald I. Eisenstein (Reg. No. 30,628)	Brian L. Michaelis (Reg. No. 34,221)
David G. Conlin (Reg. No. 27,026)	Henry D. Pahl, Jr. (Reg. No. 20,438)	Cara Z. Lowen (Reg. No. 38,227)
George W. Neuner (Reg. No. 26,964)	Peter J. Manus (Reg. No. 26,766)	John L. Welch (Reg. No. 28,129)
Ernest V. Linek (Reg. No. 29,822)	David S. Resnick (Reg. No. 34,235)	Milton Mc.K. Oliver (Reg. No. 28,333)
Linda M. Buckley (Reg. No. 31,003)	Peter F. Corless (Reg. No. 33,860)	David D. Lowry (Reg. No. 38,538)

SEND CORRESPONDENCE TO:

David G. Conlin, Esq.
Dike, Bronstein, Roberts & Cushman
130 Water Street
Boston, Massachusetts 02109

DIRECT TELEPHONE CALLS TO:

(name and telephone number)

(617) 523-3400

201	FULL NAME OF INVENTOR	LAST NAME	FIRST NAME	MIDDLE NAME
	RESIDENCE & CITIZENSHIP	CITY	STATE OR FOREIGN COUNTRY	COUNTRY OF CITIZENSHIP
	POST OFFICE ADDRESS	POST OFFICE ADDRESS	CITY	STATE OR COUNTRY AND ZIP CODE
		HAYASHI	Yoshio	
		Tokushima	JAPAN	JAPAN
		1-8-301, Jounancho 2-chome, Tokushima	Tokushima 770	JAPAN
202	FULL NAME OF INVENTOR	LAST NAME	FIRST NAME	MIDDLE NAME
	RESIDENCE & CITIZENSHIP	CITY	STATE OR FOREIGN COUNTRY	COUNTRY OF CITIZENSHIP
	POST OFFICE ADDRESS	POST OFFICE ADDRESS	CITY	STATE OR COUNTRY AND ZIP CODE
		SUGINO	Hiromu	
		Tokushima	JAPAN	JAPAN
		1325, Nishiyama, Kamihachimanchi, Tokushima	Tokushima 770	JAPAN
203	FULL NAME OF INVENTOR	LAST NAME	FIRST NAME	MIDDLE NAME
	RESIDENCE & CITIZENSHIP	CITY	STATE OR FOREIGN COUNTRY	COUNTRY OF CITIZENSHIP
	POST OFFICE ADDRESS	POST OFFICE ADDRESS	CITY	STATE OR COUNTRY AND ZIP CODE

Signature of Inventor 201 <i>Yoshio Hayashi</i>	Signature of Inventor 202 <i>Hironu Sugita</i>	Signature of Inventor 203
Date <i>December 9, 1996</i>	Date <i>December 9, 1996</i>	Date

[illegible]

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT: Y. Hayashi, et al. EXAMINER: Unassigned
SERIAL NO. Divisional of 09/076,938 GROUP: Unassigned
FILED: Herewith
FOR: COMPOSITION CONTAINING α -FODRIN OR α -FODRIN
FRAGMENT PROTEIN

ASSISTANT COMMISSIONER FOR PATENTS
WASHINGTON, DC 20231

Sir:

TRANSFER OF SEQUENCE LISTING

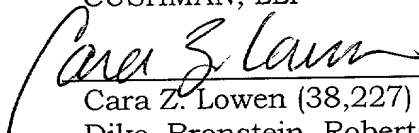
The computer readable form in this application is identical with that filed in U.S.S.N. 08/736,434, filed October 24, 1996. In accordance with 37 CFR 1.821(e), please use the computer readable form filed October 24, 1996, in that application as the computer readable form for the instant application. It is understood that the Patent and Trademark Office will make the necessary change in application number and filing date for the computer readable form that will be used for the instant application.

In accordance with 37 CFR §§1.821-1.825, I hereby state that the content of the paper, computer-readable copies of the sequence listing submitted in accordance with 37 CFR §1.821(c) and (e) on October 24, 1996, respectively, are the same. I hereby state that the submission, filed in accordance with 37 CFR §1.821(g), does not introduce new matter.

Respectfully submitted,

DIKE, BRONSTEIN, ROBERTS &
CUSHMAN, LLP

Date: Feb 8, 2000



Cara Z. Lowen (38,227)
Dike, Bronstein, Roberts
& Cushman, LLP
130 Water Street
Boston, MA 02109

Tel. No. (617) 523-3400

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Hayashi, Yoshio
Sugino, Hiromu

(ii) TITLE OF INVENTION: COMPOSITION CONTAINING α -FODRIN OR α -FODRIN
FRAGMENT PROTEIN

(iii) NUMBER OF SEQUENCES: 1

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: Dike, Bronstein, Roberts & Cushman, LLP
(B) STREET: 130 Water Street
(C) CITY: Boston
(D) STATE: MA
(E) COUNTRY: USA
(F) ZIP: 02109

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Diskette, 3.5 inch, 1.44 MB
(B) COMPUTER: IBM PC
(C) OPERATING SYSTEM: Dos 5.0
(D) SOFTWARE: WordPerfect 5.1

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER: Unknown (Express Mail #TB447318300US)
(B) FILING DATE: 10-24-96
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: 100765/1996
(B) FILING DATE: 04-23-1996

(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: David S. Resnick, Esq.
(B) REGISTRATION NUMBER: 34,235
(C) REFERENCE/DOCKET NUMBER: 46,910

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: 617-523-3400
(B) TELEFAX: 617-523-6440
(C) TELEX:

(1) INFORMATION FOR SEQ ID NO: 1

(i) SEQUENCE CHARACTERISTICS

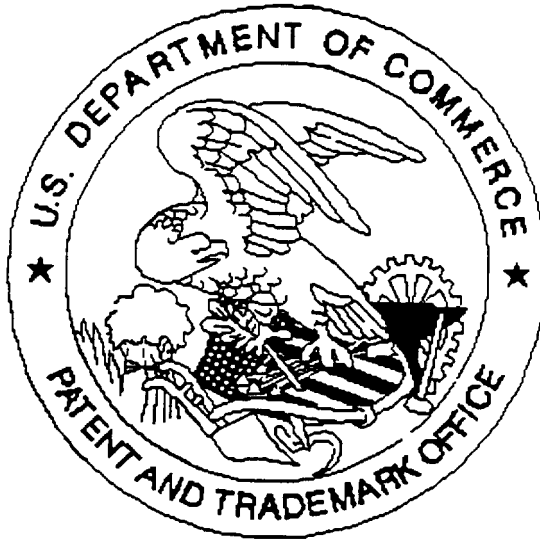
(A) LENGTH: 20
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULAR TYPE: peptide

(iii) SEQUENCE DESCRIPTION: SEQ ID NO: 1

Arg Gln Lys Leu Glu Asp Ser Tyr Arg Phe Gln Phe Phe Gln Arg
1 5 10 15
Asp Ala Glu Glu Leu
20

United States Patent & Trademark Office
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